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ANIMAL VACCINES.

BY

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PREFACE.

No claim of originality is made in respect of any of the material contents of this paper. Its framework is composed of notes taken at Dr. Roux's course of lectures at the Institut Pasteur during the Session of 1893, supplemented by information gleaned at the demonstrations of Professor Schütz and Dr. Koch in Berlin. The great bulk of information has, however, been derived from current home and foreign literature. The fullest references have been given, and in every case the utmost has been done, by referring to original sources, to ensure this paper reflecting accurately the latest views on its particular subject. If the work has called for some labour the writer will be amply rewarded by feeling that he has in any degree assisted the study of this branch of veterinary science.

ANIMAL VACCINES.

By J. A. W. DOLLAR, Esq.

For the purposes of this paper I shall regard the term Vaccine as including all substances resulting from the life activities of micro-organisms (including such organisms themselves) which may be employed for the purpose of conferring immunity against inoculation or infection. Certain Vaccines are also capable of ameliorating or curing existing disease, though this is a therapeutic and not a vaccinal action. The above definition excludes the vaccinal use of blood of congenitally immune animals, unless after artificial preparation; but as considerable doubt seems to rest on such claims, and as the above already covers a large section of all bacteriological research, I make no apology for its exclusiveness. Nor do I attempt to excuse the use of the word Vaccine, though I am aware exception has been taken to it—relying, rather, for justification on the precedent established by Pasteur, who, at the London International Medical Congress of 1881, said: "I have lent to the expression vaccination an extension which I hope science will consecrate as a homage to one of the greatest men of England—Jenner."

In the following pages I hope to set before you a brief account of

(1) The Origin of Protective Inoculation.

(2) The Methods of Conferring Immunity against the Common Diseases of Animals.

(3) The Views held as to the Nature of Immunity.

HISTORY OF PROTECTIVE INOCULATION.

It is matter of common knowledge that in certain infective diseases one attack seems to guard against a second. This is the basis of all protective inoculation. Whatever the means employed, the end is the same—viz., to produce a modified and benignant form of disease which shall leave the system proof against the original malady. This great principle underlay the work and teaching of Jenner, just as it later underlay the experiments of Pasteur.

But neither Jenner nor Pasteur was original in recognising the fact, for it had been known and, practically, utilised for hundreds of years previously.

During the middle ages smallpox proved a veritable scourge in Europe, carrying off in its periodical progresses hundreds and thousands of lives. Its ravages naturally stimulated the search for some antidote, and it was very early found that inoculation produced a form of disease which, while less severe than usual, yet conferred full protection. The Turks, in particular, regularly resorted to it, as was pointed out in 1718 by Lady Mary Montague, in one of her famous letters, and so impressed was she with the value of the precaution, that she submitted her own children to it. Writing from Belgrade, on the 23rd March, 1718, she says: "The boy was ingrafted last Tuesday, and is at this time singing and playing, very impatient for his supper; I pray God my next may give as good an account of him."

The child did well, and when her ladyship returned to England she particularly drew attention to the benefits of the operation. King George authorised the inoculation of six prisoners in Newgate, and then of six orphans. These cases being successful, the Princesses of the Royal Family and more than two hundred other persons were inoculated. Certain unsuccessful cases, however, combined with the opposition of the clergy, discredited the operation, though in France its use continued until the great discovery of vaccination by Jenner.

Vaccination signalised a new era. Not that it was so important a pathological advance, nor that it revealed an absolutely new principle, but that it replaced an unreliable and dangerous process by a certain aud safe one.

Called first attention to Jesty

Both in this country and in France and Germany it had been known that milkmaids who had been inoculated with cowpox (French "picote") were afterwards proof against smallpox, and it is related in a most exhaustive paper on "Inoculation," read to this Association by the late Mr. Archibald Robinson, of Greenock, that a certain Mr. Jesty, of Downshay, in the Island of Purbeck, had taken cognisance of the fact, and, applying it practically, had in 1774 inoculated his wife and two sons with cowpox. So successful was the operation that, though the family was frequently exposed to smallpox during a period of thirty-one years, none of them contracted the disease. The facts are vouched for by a number of physicians and surgeons. Mr. Jesty seems, therefore, to have been first in the field; but we are altogether indebted to the untiring energy and splendid devotion of Jenner for the general adoption of vaccination.

It will be seen that thus far advances in the direction of protective inoculation had been distinctly empirical. Inoculation aimed at producing a mild attack of smallpox. Vaccination had as its basis the observation that cowpox was transferable to the human subject, that it ran a benign course, and conferred immunity from smallpox.

But the nature of smallpox was not yet understood. Its causative agent was unknown. The minute changes which occurred in the bodies of the diseased were unrecognised, and naturally no explanation could be given of the action of vaccination. For a whole century medicine has been unable to derive anything from Jenner's discovery beyond its actual application, which is the one result achieved. Cowpox is a malady belonging exclusively to a race of animals. Man can only observe it; he cannot create it. Suppress cowpox and there will be no more vaccination.

Pasteur's (I.) coming, then, marked the third great epoch in the history of our battle with disease.

In his discoveries the deadly virus serves as a starting-point for the vaccine. The hand of man plants it, his art propagates it independently of animals, he increases its venom or mitigates it at will, and, finally, turning it from its deadly nature, "plucks from this nettle danger the flower safety."

Jenner had established a great fact, Pasteur established a great principle.

The early life of the great French scientist was devoted to chemistry. He afterwards took up the study of fermentation and the diseases of wines and beers. From this he was led on to investigate the diseases of silkworms, and his first essay in the field of animal pathology was made in connection with fowl cholera.

After dwelling long on Jenner's discovery, this question arose in Pasteur's mind: If contagious maladies do not repeat themselves, why should there not be found for each of them a disease which, whilst differing from, yet has some likeness to them, and which, acting in them as cowpox does in smallpox, would have the virtue of a prophylactic? The elements of the virus are living beings, which can be kept in artificial cultivations; and, as they are only distinguished from other lowly-organised beings and plants by their property of invading the bodies of men and animals, the question naturally presents itself: Would it not be possible to modify them by cultivation, just as other plants are modified? A chance occurrence, one of those chances which not infrequently occur to those who are steadfastly looking out for them, opened out to Pasteur the way to a discovery which may well be classed amongst the greatest of the age.

In studying chicken cholera M. Pasteur struck on the means of obtaining an attenuated virus. This disease is caused by small cocci, about 2 to 3 micromillimetres in diameter. When these are injected into the connective tissue of a fowl the animal dies next day with characteristic symptoms of diarrhœa and exhaustion. Old cultivations of these cocci, however, not only do not produce death, but confer immunity against subsequent inoculation with virulent cocci (II.).

By causing this microbe to pass from culture to culture, in an artificial medium a sufficient number of times to render it impossible that the least trace of the virulent matter from which it originally started should exist in the last cultivation, Pasteur furnished the absolute proof that infectious microbes are the sole

I. "Louis Pasteur." His Life and Work. By his Son-in-law. 1883.
II. Pasteur, sur le cholera des Poules. Comptes Rendus, 1880.

authors of the diseases which correspond to them. This culture may be repeated ten, twenty, a hundred, even a thousand times; in the last culture the virulence is not extinguished, nor even sensibly weakened. But it is a fact, worthy of attention, that the preservation of the virulence in successful cultures is assured only when no great interval has been allowed to elapse between the cultures. For example, the second culture must be sown twenty-four hours after the first, the third twenty-four hours after the second, the hundredth twenty-four hours after the ninety-ninth, and so on. If a culture is not passed on to the following one until after an interval of several days or several weeks, and particularly if several months have elapsed, a great change may then be observed in the virulence. This change, which generally varies with the duration of the interval, shows itself by the weakening of the power of the contagium. If the successive cultures of fowl cholera, made at short intervals, have such virulence that ten or twenty inoculated birds perish in the space of twenty-four or forty-eight hours, a culture which has remained, say, for three months in its flask, the mouth of which has been protected from the introduction of all foreign germs by a stopper of cotton wool, which allows nothing but pure air to pass through it—this culture, if used to inoculate twenty fowls, though it may render them more or less ill, does not kill any one of them. After some days of fever they recover both their appetite and spirits. And, further, if new cultures be made from the mitigated virus, none of the former virulence returns. The daughter cultures have exactly the same effect upon fowls as the mother culture had when it furnished the seed. The new property of the virus, viz., that of harmlessness for those animals to which it was previously so fatal, can be perpetuated for successive generations.

But, if this phenomenon is extraordinary, here is one which is surely as singular, though in a different sense. If, after the cure of these twenty birds, they are re-inoculated with a very virulent virus—that, for instance, which has just been mentioned as capable of killing its hundred per cent. of those inoculated with it—those fowls would become rather ill, but they would not die. The conclusion is simple: the disease can protect *from itself*. It has evidently that characteristic of all virulent diseases, that it cannot attack a second time.

However curious they may be, the above principles are not things unknown in pathology. The great novelty which is the outcome of the preceding facts, and which gives them a distinctive place in our knowledge of virulent diseases, is tnat we have to deal with a disease of which the virulent agent is a microscopic parasite, a living organism, cultivated outside the animal body, and that the attenuation of the virulence is in the power of the experimenter. He creates it, he diminishes it, he does what he will with it; and all these variable virulences he obtains from the maximum virulence by manipulation in the laboratory. Looked at in juxtaposition with the great fact of vaccination for smallpox, this weakened microbe, which does not cause death, behaves like a real vaccine relatively to the microbe which kills, producing a malady which may be called benign, since it does not cause death, but is a protection from the same malady in its more deadly form. (I.)

The attenuated virus therefore discovered by M. Pasteur is quite as efficacious against chicken cholera as Jenner's vaccination against smallpox. But while we are ignorant of the relations between smallpox and vaccine, none of the relations between the vaccine virus and the virulent virus of chicken cholera are hidden from us.

ANTHRAX, SPLENIC APOPLEXY, SPLENIC FEVER, ANTHRAX FEVER, MALIGNANT PUSTULE OR CARBUNCLE, CARBUNCULAR FEVER, GLOSSANTHRAX, BLAIN.

Fr.: CHARBON, PUSTULE MALIGNE, FIEVRE CHARBONNEUSE, SANG DE RATE, MALADIE DU SANG. *Ger.*. MILZBRAND, KOHLENBEULE. *Ital.*. ANTRACI.

Anthrax is produced by the presence in the body of large motionless aerobic bacilli, about five to twenty micromillimetres long and 1 to 1·5 m.m. broad. Their ends appear squarely cut off, and sometimes a little swollen, so that when two or more lie together they resemble a section of bamboo, the ends of the bacilli corresponding to the joints. They occur singly or in long chains. Under

1. Roux, Croonian Lecture, 1889.

certain conditions (such as exhaustion of the nutritive fluid) small bright highly refractile spots appear in their length. These increase in size, the intervening portions of the bacillus degenerate, and the little bodies, which are termed spores, are now set free.

In 1849, Pollender, and in 1850, Rayer and Davaine (I.) described these organisms in animals, and again in 1857, Brauell (II.), examining the blood of a man affected with anthrax, found the same bacillus. But none of these observers seem to have appreciated its exact significance.

In 1863 Davaine (III.) commenced a series of observations on anthrax, which, carried on until 1873, almost conclusively proved that the anthrax bacillus was the *causa causans* of the disease. This point was finally settled by Koch (IV.) in 1876, who gave a most exact description of the characters of the bacillus, and drew attention amongst other points to the spores and their remarkable qualities. Pasteur and his pupils, Joubert, Roux, and Chamberland next succeeded in obtaining pure cultures of the bacillus, and in reproducing the disease by means of such cultures.

Pasteur showed that the virulence of these bacilli could not be diminished by exposure to the atmospheric oxygen, as was that of fowl cholera. This he considered due to the presence of spores which were formed during the first hours of cultivation. He says (V.): "In order, therefore, to repeat the conditions analogous to those which were successful in chicken cholera, we must first prevent the bacilli from forming spores. The way to do this is by cultivating anthrax not now at a temperature of 35°C., but of 42°C. to 43°C. Under these conditions the bacilli develop, producing the filaments, but no spores. If we try every three days, for example, the intensity of the virulence of a culture thus made at a high temperature, and in which no spores are formed, by inoculating it into sheep and rabbits, we shall find that in the first days of the experiment all the inoculated animals succumb; then that the virus becomes less active, and the sheep resist, while the rabbits still die, though after a more or less prolonged illness. After a still longer period the culture made at 42°C. loses its danger for the rabbit, but is still fatal to guinea-pigs and mice. Finally, it is quite harmless even to these little rodents, very sensitive to the virus as they are. It is, therefore, only necessary to draw off each day a little of the culture from the bottle at 42°C., and to place it in bouillon at 35°C. in order to have a series of cultures of graduated virulence, and furnished with spores capable of giving each of these special degrees of intensity."

The sheep and oxen which receive these attenuated bacteria exhibit a passing fever, but if later on one inoculates them with virulent virus it has no effect. One has, therefore, only to choose among the degrees in this scale of virulence that which will give to the animal one is desirous of protecting from anthrax an illness which, whilst slight, is sufficient to ensure the exemption desired. In practice, the vaccinations of oxen and sheep are done twice. The virus of the first inoculation is very attenuated, and is intended to prepare the animal for the action of the second and more energetic inoculation, which is prepared twelve days after the first. The whole difficulty in anthrax inoculation consists in the choice of these two viruses, and in keeping the relation between their degrees of virulence invariable. The following table shows the results of inoculation in France from 1882 to 1893 (VI.):—

I. Rayer, Inoculation du sang de rate (Mémoires de la Société de biologie, 1874. p. 141).

II. Brauell, Versuche und Untersuchungen betreffend den Milzbrand der Menschen und Thiere Virchow's Archiv., 1857, XI., p. 131).

III. Davaine, Recherches sur les infusoires du sang dans la maladie connue sons le nom de sang de rate (Comptes Rendus, 1865, vol. lvii., pp. 320, 351, and 386).

IV. Koch, Die Ætiologie der Milzbrand-Krankheit begründet auf die Entwickelungsgeschichte der Bacillus Anthracis (Cohn's Beiträge Zur Biologie der Pflanzen, vol. ii., p. 277, 1876.

V. Croonian Lecture, 1889.

VI. "Annales de l' Institut Pasteur," March, 1894, p. 162.

YEARS.	Total Number of Animals Vaccinated.	Number of Veterinary Reports.	Animals Vaccinated and reported by Veterinary Surgeons.	MORTALITY.			Total.	Total Loss per cent.	Average loss before discovery of Vaccination
				After the 1st Vaccination	After the 2nd Vaccination	During the rest of the year.			
SHEEP.									
1882	270,040	112	243,199	756	847	1,037	2,640	1·08	Per cent. 10
1883	268,505	103	193,119	436	272	784	1,492	0·77	,,
1884	316,553	109	231,693	770	444	1,033	2,247	0·97	,,
1885	342,040	144	280,107	884	735	990	2,609	0·93	,,
1886	313,288	88	202,064	652	303	514	1,469	0·72	,,
1887	293,572	107	187,811	718	737	968	2,423	1·29	,,
1888	269,574	50	101,834	149	181	300	630	0·62	,,
1889	239,974	43	88,483	238	285	501	1,024	1·16	,,
1890	223,611	69	69,865	331	261	244	836	1·20	,,
1891	218,629	65	53,640	181	102	77	360	0·67	,,
1892	259,696	70	63,125	319	183	126	628	0·99	,,
1893	281,333	30	73,939	234	56	224	514	0·69	,
TOTALS	3,296,815	990	1,788,677	5,668 (0·32)	4,406 (0·24)	6,798 (0·35)	16,872	0·94	—
OXEN.									
1882	35,654	127	22,916	22	12	48	82	0·35	Per cent. 5
1883	26,453	130	20,501	17	1	46	64	0·31	,,
1884	33,900	139	22,616	20	13	52	85	0·37	,,
1885	34,000	192	21,073	32	8	67	107	0·50	,,
1886	39,154	135	22,113	18	7	39	64	0·29	,,
1887	48,484	148	28,083	23	18	68	109	0·39	,,
1888	34,464	61	10,920	8	4	35	47	0·43	,,
1889	32,251	68	11,610	14	7	31	52	0·45	,,
1890	33,965	71	11,057	5	4	14	23	0·21	,,
1891	40,736	68	10,476	6	4	4	14	0·13	,,
1892	41,609	71	9,757	8	3	15	26	0·26	,,
1893	38,164	45	9,840	4	1	13	18	0·18	,,
TOTALS	438,824	1,255	200,96	177 (0·09)	82 (0·04)	431 (0·21)	691	0·34	—

It will be seen from these figures that whereas the mortality among sheep before preventive inoculation was 10 per cent., it has now fallen to less than 1 per cent.

The present would scarcely be a fitting time or place for entering into the many questions which have been raised relative to the efficiency of this inoculation, even if such could be maintained in face of the above figures; but I may be allowed to quote the opinions expressed by Professors McFadyean and Sims-Woodhead, in a paper formerly read to this Association. They considered that an impartial review of all the evidence justified the following conclusions:—

"(1) That Pasteur has succeeded in preparing a vaccin, by the employment of which domestic animals are put in possession of a high degree of immunity against spontaneous or inoculated anthrax.

"(2) That by no known method can there be obtained a vaccin of absolutely uniform strength.

"(3) That it is not possible to obtain a vaccin that is at once and equally applicable to all the different species of domestic animals, nor even to all the different breeds of the same species.

"(4) That even in the most capable hands accidents, capable of entailing serious results, may happen in the preparation of the vaccin or in its employment."

The six years' experience acquired since this was written shows that the latter conclusion may be somewhat modified. According to the statistics of the Pasteur Institute, inoculation for anthrax has now a much lower mortality than the operation of castration. In addition to Pasteur's methods of preparing a protective fluid, others have been suggested. Thus Tonssaint (I.) adopted the method of rapidly heating defibrinated anthrax blood to 55°C., and maintaining that temperature for ten minutes. With this material he conferred immunity on sheep. His method, however, proved unreliable, because, as M. Chauveau pointed out, the heat did not act equally well on all parts of the vaccine material at the same time. M. Chauveau (II.) then undertook a series of experiments, in which he first employed heat and afterwards compressed oxygen as the mitigating agent. He found that the greater the heat the more rapid the diminution in virulence. Thus, whilst a cultivation at 45°C. requires several days to become sufficiently attenuated, the same result can be produced in a few hours at 47°C., and in a few minutes at from 50°C. to 53°C. Chauveau considered this action in no way dependent on the air, but simply on the temperature. Tonssaint also found that a modified virus might be produced by exposing the bacilli to the action of various antiseptics, which prevented the formation of spores, and Roux and Chamberland have confirmed this. Chauveau (III.) obtained a rapid attenuation of the bacilli by employing compressed oxygen, and Arloing (IV.) has found that the virulence is diminished, and finally lost, by exposure to the direct solar rays.

Various experimenters have found that the physiological activity of the anthrax bacillus is capable of being modified by cultivation in the bodies of particular species of animals. Thus, Klein (V.) found that blood taken from a white mouse dead of anthrax is a protective vaccin for sheep; while Sanderson and Duguid think that blood of a guinea-pig dead of anthrax is modified for cattle. (Notes on some of the Micro-Parasites of the Domesticated Animals, 1888, Professors McFadyean and Sims-Woodhead.) MM. Chamberland and Roux prepare a vaccine by adding from ·2 to ·4 per cent. of bichromate of potash, or ·125 per cent. of carbolic acid to the cultivating medium. M. Gamaléia gives an account of some experiments by this method in the October number of the *Annales de*

I. Tonssaint, De l'immunité pour le charbon acquise à la suite d'inoculation préventive. (Comptes Rendus, 1880, XCI., pp. 135 and 303.)

II. Chauveau. (Comptes Rendus de l'Académie des Sciences, 1882, XCIV., p. 1694, and 1883, XCVI., p. 553.)

III. Chauveau De l'atténuation des cultures virulentes par l'oxygène comprimé. (Comptes Rendus, 1881, XCVIII., p. 1232, and 1885, C., p. 320.

IV. Arloing, Influence de la lumière blanche et de ses rayons constituants sur le développèment et les propriétés du bacillus anthracis. (Archives de Physiologie, 1886, Vol. VII., p. 209.)

V. Reports of Medical Officer Local Government Board, 1882.

l'Institut Pasteur (1888). Of twenty-two sheep inoculated, three died of anthrax—the rest proved protected against the most active anthrax virus.

Roux and Chamberland (I.) have also shown that it is possible to obtain a fluid which protects sheep from virulent anthrax by filtering anthrax cultures through porcelain.

In 1889 Professor Hueppe and Dr. Cartwright Wood (II.) published an account of some experiments they had made in regard to the immunity-conferring powers of a certain bacillus they had met with in samples of impure water.

This organism closely resembled the anthrax bacillus in form and method of growth, but differed from it in producing no disturbance when injected into mice. On further investigation, it was found that such inoculated animals proved in a varying degree resistant to anthrax. Some resisted for eight to eleven days, and their blood then furnished cultures which required three days to kill unprotected mice, instead of the normal period of twenty hours. In addition to this, some mice proved immune even against repeated treatment with the most active anthrax virus. Considering the excessive susceptibility which mice exhibit towards anthrax, this discovery is of the highest importance, and may point to a simpler method of rendering the higher animals immune.

In November, 1890, Blagovestchensky (III.) published some interesting facts regarding the protective action of the bacillus pyocyaneus in cases of anthrax. Emmerich (IV.) had previously shown that the streptococcus of erysipelas, when injected under the skin, exerted an effect antagonistic to that of anthrax. He found that when the streptococcus was injected before inoculation with anthrax the animal was often protected, and that, even if the employment of the streptococcus were delayed until after local symptoms of anthrax had appeared, many cases were still saved. Emmerich, therefore, concluded that the streptococcus of erysipelas antagonises the bacillus of anthrax. Watson Cheyne (V.) generally confirmed this. Pavlovsky (VI.) had shown that the bacillus of Friedlander and the micrococcus prodigiosus also counteract the action of bacillus anthracis. M. Bouchard (VII.) stated that the bacillus pyocyaneus possesses the same property.

Guignard and Charrin (VIII.) cultivated the bacillus pyocyaneus and the bacillus anthracis together, and found that animals succumbed much later than usual when inoculated with bacillus anthracis so treated.

Woodhead and Cartwright Wood (IX.) were, in some cases, able to save animals inoculated with anthrax by injecting sterilised cultures of blue pus.

Several other workers confirm—and it must be admitted as a demonstrated fact—that one bacillus may exercise a protecting influence against another. The question then arises, How is this action produced? Emmerich and Freudenreich have stated that the bacillus anthracis grows vigorously in the the presence both of the bacillus pyocyaneus and of the streptococcus of erysipelas in artificial cultures and from their experiments it therefore seems that the protective influence is in some way dependent, not on the organisms directly antagonising each other, but on their re-acting through the living body. The experiments, however, made by Blagovestchensky point rather in the other direction. He sets forth his researches in the paper before alluded to, and concludes, "The simultaneous inoculation of pyocyanic and anthrax bacilli into the aqueous humour of the eye is generally prejudicial to the development of the latter, but, except very occasionally, does not protect from subsequent anthrax infection. . . The life products of pyocyanic bacilli are in part volatile . . and even outside the organism markedly impede the development of anthrax bacilli."

I. Roux and Chamberland (Annales de l'Institut Pasteur, August, 1888, p. 405).
II. Lancet (7th December, 1889).
III. Annales de l'Institut Pasteur, 1890, p. 689.
IV. La guérison du Charbon, Arch. f. Hygiene, Vol. VI., p. 442.
V. London Medical Record, 1887.
VI. De la guérison du charbon par les bactéries et de la manière d'être du charbon dans l'organisme. Virchow's Archiv., 1887, Vol. CVIII., p. 494.
VII. Comptes Rendus Ac. des Sc., April, 1888.
VIII. Action du bacille pyocyanique sur la bactéridie charbonneuse (Comptes Rendus, Apl., 1889).
IX. De l'action antidotique exercée par les liquides pyocyaniques sur le cours de la maladie charbonneuse (Comptes Rendus, vol. cix., No. 26, 1889).

Professor Fodor (I.), of Buda-Pesth, administered large doses of bicarbonate of soda to rabbits, and half an hour after gave an intravenous injection of anthrax virus. Further doses of sod. bicarb. were given three times a day. Only one-sixth of the animals so treated contracted anthrax, though many died from gastric catarrh, etc.; more than one-third, however, entirely recovered; while other animals to which the same quantity of culture was administered invariably died from anthrax. It would therefore seem that the free exhibition of alkalies might possibly be of service in cases of anthrax.

M. J. de Christmas (II.) treated anthrax blood (rabbit's) with eucalyptus essence, filtered it, and obtained a substance which protected rabbits from anthrax. The blood of such rabbits yields a substance which, added to bouillon, prevents the growth of anthrax bacilli in the latter.

In 1892, Hankin and Westbrook (III.) obtained a protective albumose from anthrax bacilli grown in pure peptone. This sometimes protected mice perfectly, but was sometimes only partially successful.

Lazarus and Weil (IV.) unsuccessfully attempted to confer immunity against anthrax by injecting defibrinated hen's blood, it being well known that fowls are themselves normally insusceptible to anthrax. They concluded from this that the blood serum of an animal possessing congenital immunity to anthrax cannot serve to confer immunity on an unrefractory animal. Scheuerlen doubts the correctness of this conclusion, and draws attention to the fact that Lazarus and Weil should first have administered an injection of virulent anthrax to hens whose blood they desired to employ. For as in tetanus, swine erysipelas, influenza, etc., the serum of an animal protected against a certain disease does not continuously possess the protective power, but the antitoxin material is only formed after the microbe of the disease has entered the body. Reception of the infective organism determines the formation of the protective material in the serum. The experiments of Lazarus and Weil are therefore not conclusive.

In the foregoing portion of this subject it has been shown how the virulence of the anthrax bacillus was diminished so as to render it inocuous even to mice, By an appropriate method of treatment this property, which is diminished and not destroyed, can again be restored in all its power.

Thus, the virus is incapable of killing adult mice; but let us inoculate with it a very young mouse, only one day old. This young mouse will be much more sensitive to the action of the virus than the older one, and will die in a few days. In developing itself in this young mouse the bacillus will have recovered a portion of its old fitness for life in living surroundings, and the blood of this first mouse introduced into that of one a little older will cause its death; and thus, proceeding step by step from the youngest mouse to the oldest, it will gain power to kill first old mice, then guinea-pigs, rabbits, sheep, and last of all oxen, which, among herbivorous animals, are the most indifferent to the action of anthrax.

Thus we see that we can increase the virulence as easily as we can diminish it, and that it manifests itself by the increasing power of the microbes to germinate in the bodies of living animals, a power which can thus be either acquired or lost under respectively appropriate conditions.

SYMPTOMATIC ANTHRAX, QUARTER EVIL, BLACK QUARTER. *Fr.*: CHARBON SYMPTOMATIQUE, CHARBON ESSENTIEL DE CHABERT, MALADIE DE CHABERT. *Ger.*. RAUSCHBRAND, RAUSCHENDER MILZBRAND, GERAUSCH, RAUSCH, BRAND-RAUSCH, SCHWARTZER BRAND, KALTER BRAND, SCHENKEL—HINTER—BRAND, KARBUNKELKRANKHEIT, MILZBRANDEMPHYSEM.

The bacillus of symptomatic anthrax (bacillus Chauvæi) is small, five to fifteen micromillimetres long and three thick, that is to say, as long as a red blood-corpuscle is wide. The bacilli consist of rods with rounded ends which execute rapid movements, and usually exhibit at one extremity (which is swollen out) a brilliant spore-like body. Ehlers has described various developmental forms. The bacilli occur most abundantly in the affected subcutis and in the muscles, in the gall and in the intestinal contents (Kitt), but seldom in the blood, and there-

I. See Note, Journal of Comp. Pathology and Therapeutics, Dec. 1890, p. 371.
II. Annales de l'Institute Pasteur, 1891, p. 487.
III. Ibid. 1892, p. 633.
IV. Berliner Thierärztliche Wochenschrift, June 22nd, 1893.

fore inoculation with the latter is generally without result, though after the carcase has lain for some time they may be found in the blood also. They are anaerobic. Arloing, Cornevin, and Thomas have shown that this bacillus is the cause of the disease. It is exceedingly resistant to external influences. When dried it preserves its properties better than when moist, and will remain active for as long as two years. Burial of the carcase does not destroy the bacillus even after six months, and it is quite unaffected by putrefactive organisms, by anthrax bacilli, or by low temperatures.

Long before the researches of the above experimenters it had been known that animals which survived one attack were proof, for a long time at least, against a second, and that an immune cow generally transmitted the same characteristic to her offspring. It is also a fact that sucking calves are relatively immune. Messrs. Arloing, Cornevin, and Thomas therefore attempted to confer immunity by introducing a minimal quantity of virulent muscle-plasma in the subcutaneous tissue. It was found that one-tenth of a drop of active muscle-plasma always proved fatal, but that a smaller quantity sometimes produced no effect, or only a passing illness, followed by recovery. In the latter case the animal was proof against injection with relatively large quantities of virulent material. Here is an example (I.).

"6th Feb., 1881. A sheep was inoculated in the cellular tissue of the tail with one-fifteenth of a drop of muscle-juice mixed with four drops of water. The 7th and 8th, slight malaise, temperature raised four-tenths of a degree.

"10th Feb. The animal is again lively and well, eats well, health is completely restored.

"11th Feb. Half a centimetre of fresh, very virulent muscle-juice was injected; no bad effects either local or general, whilst a guinea-pig inoculated at the same time and with the same fluid died in thirty hours."

This method proving too uncertain the experiment was made of injecting from five to ten drops of virulent material directly into the veins. The animals suffered a passing fever and were protected. But as very great danger exists of transferring some of the bacilli to the subcutaneous tissue covering the vein whilst withdrawing the syringe, it was found necessary to dissect out the jugular before inserting the point of the syringe. This and other objections led to a search for fresh methods of protection. In May, 1881, some sheep were treated by injecting four to five c.c. of virulent liquid into the trachea by a special form of syringe. Eight days, and again eleven days later, these sheep were inoculated with virulent material and survived—the control animals dying. As, however, this method required just as many precautions as intravenous injection, it was not proceeded with beyond the laboratory stage.

Further experiment showed that the simplest and most efficient method consisted in inoculating the animals in the tail with a virus modified by heat. Though in this region the vaccin only produces a slight and temporary swelling, it protects against the disease with great certainty. This benign character of the local reaction is thought to be due to the density of the connective tissue of the part, and especially to its temperature, which is much lower than in other parts of the body. Both of these factors impede the rapid development of the bacillus.

Experiments made during the past few years in France, Switzerland, Austria, and Germany show conclusively that this vaccination greatly lowers the death-rate of symptomatic anthrax. The vaccine is prepared as follows:—40 grammes (1 part) of affected muscle are dried at a temperature of 32° C., then intimately mixed with 80 grammes (2 parts) of water. The mixture is divided into 12 parts of 10 grammes each, and each part placed in a flat dish. Six of these are dried in the thermostat at 100°C., and six at 80°C. for six hours. The first series give a feeble virus—the first vaccin, the others form the second vaccin. Prepared in this way the material keeps for a considerable time. The first vaccination is made by taking one-tenth of a gramme of the material dried at 100°C. and mixing it intimately with 5 grammes of water by trituration. This fluid is filtered through fine linen and injected at the rate of one-half cubic centimetre

1. "Le Charbon symptomatique du Boeuf," Arloing, Cornevin, and Thomas, Paris, 1887, p. 113.

per animal. The first inoculation is made on the under surface of the tail three hand-breadths from its free extremity. The hair should first be removed. A fine trochar is introduced from below upwards, between the skin and the bone, for a distance of 8 centimetres, the trochar withdrawn, and the point of the syringe inserted through the same aperture. The contents of the syringe must first be shaken, so as to distribute the solid contents evenly throughout the fluid. The vaccin is then slowly injected Should the puncture be followed by hæmorrhage one must wait till this stops or make a fresh opening.

The second inoculation follows ten days after the first. The manipulation is the same, but the spot chosen is only two hand-breadths from the free end of the tail. The end of winter and the spring are the best times for inoculating.

Cornevin vaccinated 125 animals in France in 1883. In Switzerland most of the operations in 1884 were made by Hess and Strebel. The number inoculated was 2,oco, and they were all placed on pasturages where the disease abounded. Amongst the animals vaccinated, the mortality was only one twenty-eighth of that in the non-vaccinated, or in other words twenty-eight non-vaccinated animals died from the disease for every vaccinated one. (Strebel.) In 1885, 15,137 animals were vaccinated in Canton Bern alone. The results were very satisfactory. In Austria, Sperk vaccinated 925 animals during the year 1885. They were then placed on notoriously infected pastures. The results were excellent. None died after vaccination, though three calves succumbed to the first injection. In 1886, 2,140 young oxen were vaccinated in the Salzburg district, and 3,820 in Tyrol-Vorarlberg. The losses were four in the former and six in the latter case. The mortality among non-vaccinated animals was from 10 to 20 per 1,000, and among those vaccinated from 2 to 4 per 1,000.

In 1888, 2,086 animals were vaccinated in the districts of Gruyère, Singine Sarine, Glaue, Veveyse et Lac ; of these only three died of the disease during the year, *i.e.*, ·14 per cent. The returns for Switzerland show that between the years 1884 and 1888, 8,641 animals were treated, and only 15 died of the disease. (·17 per cent., or 1 in 576.) Of 21,000 non-vaccinated oxen under precisely the same conditions, 491 (2·34 per cent., or 1 in 43) died of the disease. A comparison shows that nearly 14 times more non-vaccinated animals died than vaccinated.

Kitt was able to protect oxen and sheep by injecting them with a virus prepared at a temperature of 85 to 90°C. for six hours, or which had been exposed for a short time to steam heat. This is about equal in strength to the second vaccine of the French experimenters. It is without danger to oxen and may be injected into the subcutaneous tissue of the shoulder. A dose ten times as great as that required to give immunity was found to have no bad effect, and the local changes were insignificant.

In 1888 Roux (I.) vaccinated some guinea-pigs by injecting bouillon cultures sterilised by heating to 115°C., or by filtration through porcelain. Three doses of 40 c.c. of the fluid are injected at intervals of two days. Guinea-pigs so treated successfully resisted injection with a solution of virulent symptomatic anthrax powder and lactic acid, which killed the control animals in twenty-four hours.

In 1890, 5,587 young cattle were inoculated in six districts of Canton Freiberg (Switzerland.) Of these 14 died of black quarter, *i.e.*, about ·25 per cent., or 1 in 398. Arloing and Cornevin's vaccin was used.

In 1891 (II.) a small number of experiments were made in this country, both by the method of Arloing and Cornevin, and by that of Kitt. The Midland Counties Veterinary Medical Society appointed a committee and devoted a considerable sum of money to investigating the value of the above methods, but the published results were so remarkable, and in fact inexplicable, that no trustworthy conclusion can be drawn from them.

Kitt (III.) has latterly employed pure cultivations of the bacillus of symptomatic anthrax in inoculating against that disease. Arloing and Thomas found great difficulty in cultivating the bacillus, and there is little doubt that many of the irregularities in the action of their vaccin are due to the presence of accidental

I. Roux, Annales de l'Institut Pasteur, Feb. 1888, p. 49.
II. See Journal of Comparative Pathology and Therapeutics, 1891, pp. 53, 344, and 379.
III. Kitt, Monatshefte für Thierheilkunde V. 1, 1893.

organisms in the muscle-juice from which it is prepared. In 1889, Kitasato discovered a method of cultivating the organism, which Kitt has now turned to account.

Many anaerobic bacilli form spores which successfully resist high temperatures, even if continued for some time. Mature bacilli, being much more sensitive to heat, are soon destroyed. If now some bouillon and agar be sown with black-quarter muscle-juice and exposed to a temperature of 70° to 84°C. for an hour, all foreign bacilli are killed, and only the spores of the bacillus of black-quarter are left. But these will not readily develop into bacilli except they be kept from oxygen, which acts as a poison to them. Kitt, therefore, places the cultures, which now contain black-quarter spores alone, in a large vessel containing a quantity of alkaline pyrogallol solution. This rapidly takes up all the oxygen, and if the vessel be hermetically sealed and placed in an incubator at 33° to 38°C., the spores soon germinate into an abundant crop of bacilli. The tubes are then examined, any impure ones rejected, and new tubes sown from those which are considered satisfactory. As a measure of precaution this second generation is kept at 50° to 70°C., which effectually prevents any foreign bacilli multiplying in it. Once the bacillus is isolated its further cultivation is comparatively easy.

Kitt next set about preparing a vaccine from his pure growths. He found from experiment that the contents of tubes which had been planted a fortnight, and those heated to 100°C for 30 minutes, were no longer fatal when injected, but yet conferred immunity against virulent inoculation. In this way he protected a number of oxen, sheep, and guinea-pigs, all of which he afterwards tested with the most virulent black-quarter extract. Without exception they remained healthy. It was found that immunity was complete 14 days after a single inoculation of this kind. Kitt has clearly proved the possibility of protecting against symptomatic anthrax by injecting pure cultures, but he is still engaged in perfecting details. It will be seen that he has already obviated many of the difficulties of Arloing and Thomas's method, for whilst his process is more certain, it requires one injection only instead of two, and the agar culture only requires to be liquefied and mixed with sterilised water, as against the old tiresome and uncertain procedure of grinding up dried muscle-juice with water. Kitt has also tried the effect of injecting blood serum from protected animals into those to be immunised.

It may here be explained that if, say, virulent tetanus cultures be injected in gradually increasing quantities into an animal immune against tetanus, a peculiar change takes place in that animal's blood. Soon after the injection a drop of blood withdrawn from the body may be found to contain the specific bacilli, and if injected under the skin of a guinea-pig will cause that animal's death from typical tetanus; but after a variable period the bacilli disappear from the blood, and the latter is no longer fatal to guinea-pigs. On the contrary, it begins to protect them from virulent injections; and if we wait for a few days after inoculating the vaccinated animal, we find that its blood serum, far from proving injurious to guinea-pigs, gives them a well-marked immunity against previously deadly cultivations. The question naturally arises—How is this? and it has been suggested that under the influence of the presence of these bacilli the tissues of the body have secreted a substance which neutralises the poison of the bacilli. Thus the system is protected from the ill-effects of the disease. The substance so produced by the tissues is termed antitoxine, and appears to be present in considerable quantities in the blood serum. We are indebted for this discovery to Behring and Kitasato, and Kitt has lately put its principle into practice. A sheep vaccinated against blackleg was inoculated three times with virulent muscle-juice; it was then killed, its blood collected, and the serum allowed to separate, with precautions against contamination. Of this serum 40 c.c. (mixed with ·5 per cent of carbolic acid), was injected into another sheep at four different places. Eight days later this sheep, together with a vaccinated and a control sheep, was injected with 2·5 c.c.m. of virulent muscle-juice. The control animal died, the other two remained well; but the same method failed to protect guinea-pigs, though too much stress may not be laid on this fact, as the action of serum from vaccinated animals is well known to vary greatly. This method will again be referred to when considering swine erysipelas and tetanus.

Swine Erysipelas.

Fr.: Rouget, Mal Rouge, Rougeole du porc, Erysipele du porc.
Ger.: Der Stabchenrothlauf, Rothlaufseuche, Schweinerothlauf.

This disease is not common in England, and is *not* identical with that epizootic in swine in which intestinal lesions are the chief characteristic. It is said by Pasteur to be caused by a bacillus shaped like the figure 8, which is found in the spleen and lymphatic glands of swine which die of it.

Cornevin (I.) described short rods which may quite possibly be the forms of 8 seen by Pasteur and Thuillier. It was Löffler (II.) who first clearly defined the characteristics of the bacillus of swine fever, which he had succeeded in isolating and cultivating on solid media. Schottelius (III.) describes the bacillus as a fine rod, from ·6 to ·8 micromillimetres long, mobile when grown in bouillon, and closely resembling Koch's bacillus of mouse septicæmia.

In attempting to attenuate this bacillus, Pasteur early discovered that it was easily cultivated without forming spores. It is therefore especially amenable to the influence of the air, and in fact, if left long enough exposed to its action, its virulence is totally destroyed. But there is a further method of attenuation discovered by MM. Pasteur and Thuillier which is of great interest, as it shows the changes which a virus may undergo in its passage through animals of different kinds.

Rouget bacilli injected into a rabbit kills it within a few days. A small quantity of the "pulp" from the spleen of this rabbit inoculated into a second one will cause death still more quickly, and the disease can thus be passed through a whole series of rabbits. But the extraordinary point (discovered by MM. Pasteur and Thuillier) in these inoculations is that, as the strength of the virus augments for the rabbit, it diminishes for the pig; so much so, that after a sufficient number of passages of the virus have been made through the rabbit, it has become a vaccine for swine, able to confer upon them exemption from the fatal form of the malady.

Pasteur prepares two forms of this vaccin of different strengths, which, when inoculated successively into young pigs, confer immunity for one year.

The tables given below show the average mortality of the operation, the degree of protection afforded, etc., and enable a very good judgment to be formed of the value of vaccination for "Rouget."—

Years.	Total Number of Animals Vaccinated.	Number of Veterinary Surgeons' reports	Animals Vaccinated and reported by Veterinary Surgeons	MORTALITY.			Total.	Total loss %.	Average loss before Vaccination.
				After 1st Vaccination	After 2nd Vaccination	During the rest of the year.			
1886	—	49	7,087	91	24	56	171	2·41	20 %
1887	—	49	7,467	57	10	23	90	1·21	"
1888	15,958	31	6,968	31	25	38	94	1·35	"
1889	19,338	41	11,257	92	12	40	144	1·28	"
1890	17,658	41	14,992	118	64	72	254	1·70	"
1891	20,583	47	17,550	102	34	70	206	1·17	"
1892	37,900	38	10,128	43	19	46	108	1·07	"
Totals	111,437	296	75,455	534	188	345	1,067	1·45	—

I. Cornevin, Première étude sur le rouget du porc. Paris, 1885.
II. Experimentelle Untersuchungen über Schweine-Rothlauf (Arbeiten aus dem Kaiserlichen Gesundheitsamte, I., p. 46).
III. Der Rothlauf der Schweine. Wiesbaden, 1885.

Schütz (I.) and Kitt (II.) have repeated these experiments of Pasteur's with slightly different results.

In the Reports of the Medical Officer of the Local Government Board for 1877 and 1884, Klein traverses the statements of Pasteur as to the characters of the above bacillus, but as the disease known in France and Germany as "Rouget" and "Rothlauf der Schweine" respectively, is not identical with that termed in this country "Swine Fever," and in the United States "Hog Cholera," it is clear that Klein and Pasteur were describing different diseases.

Klein claims to have conferred immunity to swine fever by injecting artificial cultivations of his bacillus, but this cannot be considered satisfactorily established.

Metschnikoff (III.) described a number of interesting experiments on rabbits which had been vaccinated by Pasteur's method. The vaccination was followed by a passing fever, and when this ceased he injected virulent cultures under the skin. The blood soon afterwards contained a substance (antitoxin) which could be used to immunise other rabbits; so that if a rabbit received a dose of virulent cultivation sufficient to kill it, and at the same time an injection of blood serum from a vaccinated animal, it not only did not die, but it scarcely showed any fever or constitutional disturbance.

Lorenz (IV.) had described a similar method of protecting pigs as early as 1892. His experiments consisted in taking serum from pigs which had recovered from swine erysipelas, and injecting it under the skin of animals to be protected. He showed that if rabbits or swine be rendered immune by any known method, and then receive a subcutaneous injection of virulent cultivation, their blood in a short time comes to contain a material which Lorenz terms alexin, and which destroys the poison of virulent cultures. Once obtained, this protective substance (serum) serves to produce unlimited quantities of a similar fluid. Thus a pig is injected with several doses of serum, and becomes immune to virulent cultures. The latter are then injected in gradually increasing doses, at stated intervals. After each injection the quantity of alexin increases enormously, so that the pig's blood soon comes to have very pronounced prophylactic properties, and when injected in comparatively minute doses serves to protect other pigs. The more virulent the culture, and the more copious the injections, the more strongly protective the resulting serum. Lorenz stated that he had discovered a method of concentrating the active principle of the serum, and of preserving it for use in a mixture of 30 per cent. glycerine and 40 per cent. water.

In the Berliner Thierärztliche Wochenschrift, No. 13, 1893, further details of this process are given, and the subject is brought up to date (1894) by a long and most interesting article in the Deutsche Zeitschrift für Thiermedicin, Vol. 20, 1. Lorenz here reviews the theory on which his system is based, and gives the practical results attained up to the present time. His latest method is to give an injection of protective serum, at the rate of 1 c.c.m. serum for every 10 kg. of body weight. Two days later ·5 to 1 c.c.m. of pure virulent culture is injected, and again 12 days afterwards about double that quantity. In all, he has inoculated 294 pigs; of these 12 were already suffering from the disease, and whilst 6 survived, the other 6 died. The latter cases should not, however, be considered when judging the success of the method, as Lorenz only claims to protect, and not to cure, swine erysipelas. Excluding these cases, one sucking pig alone died, which Lorenz explains by the fact that it received too small a dose of protecting serum. In order to obtain the necessary serum, 57 other pigs were inoculated. None of these experienced any bad effect. Lorenz is now making preparations for the production of protective serum in large quantities, and hopes next year to be able to supply it at the rate of 50 pfennig (6d.) per dose for small pigs, and 1 mark (about 1s.) for large ones. He anticipates that his method will speedily come into general use, because he says it not only avoids the losses often consequent on the use of Pasteur's first vaccin, but it confers a more marked and lasting immunity.

I. Ueber den Rothlauf der Schweine und die Impfung desselben (Arbeiten aus dem Kaiserlichen Gesundheitsamte, I., p. 57.)

II. Untersuchungen über den Stäbchenrothlauf der Schweine und dessen Schutzimpfung (Centralblatt für Bacteriologie, 1887, II., p. 693.)

III. Metschnikoff (Ann. de l'Institut Pasteur, 1892. p. 289).

IV. Lorenz, Berliner Thierärztliche Wochenschrift, 1892, p. 142.

RABIES, HYDROPHOBIA. *Fr.*: LA RAGE. *Ger.*. WUTHKRANKHEIT, HUNDSWUTH, TOLLWUTH, WASSERSCHEU, LYSSA.

After all his work upon the prevention of fowl cholera, anthrax, and "rouget" had been accomplished, M. Pasteur (I.) devoted himself to the study of rabies. When in 1880 the work on this disease was begun in M. Pasteur's laboratory, the following facts concerning it were alone known: that it was an infectious disease; that the virus was contained in the saliva of rabid animals, and that it was transmitted through their bites. We knew also that the period of its incubation varied from some days to some months; and here our definite knowledge of its pathology ended. Many experiments, however, had been made on the subject, but two causes had rendered them especially difficult to carry out, and their success uncertain. Inoculation of the saliva of a rabid dog into a healthy animal does not always produce rabies, but often has no effect whatever. Among animals susceptible to the malady some only become rabid after such a lapse of time that the prolonged waiting, combined with the uncertainty of the results, puts the patience of the experimenter to a most severe test. The saliva of a rabid animal affords an untrustworthy virus, because it contains a number of different microbes, which, when introduced under the skin, together with the virus of rabies, may prevent the development of the latter, and set up inflammatory processes in consequence of their growth. The first thing to do, therefore, was to find some source of the virus of rabies uncontaminated by other microbes. All the symptoms of rabies arise from disturbances of the nervous system; hence the dea that in that system the rabic virus was specially to be found, presented itself to the mind. The previous attempts made, however, to show that the nervous system of a rabid dog was virulent were unsuccessful, because the manipulations to which the nervous matter was exposed in order to inoculate it introduced into it those other microbes which it was essential to exclude. By inoculating with absolute purity, from the spinal cord, the brain or the nerves of an animal that had died of rabies, M. Pasteur demonstrated that the true source of the virus was to be found in the nervous system. A portion of the nerve centres of a rabid dog injected subcutaneously into a healthy one will produce rabies more surely than the most active saliva. This demonstration enabled us to take a decided step forward in its study. The rabic virus being contained in the nervous centres, and the symptoms depending entirely upon that system, the idea naturally followed that the disease only shows itself when the nervous centres are attacked by the virus. Further, that the incubation period is governed by the time taken by the virus to travel from the point inoculated up to the cerebro-spinal axis, and for its development therein. If, therefore, the virus be inserted directly into the nervous system, where it has to develop, the incubation ought to be shortened, and the disease follow with certainty, because the virus can no longer be destroyed or diverted from its course during its long journey.

Experiment has fully confirmed this theory, as may be seen from the records of the first dog inoculated by trephining, in which the incubation period was reduced to fourteen days. In fact, any dog inoculated under the *dura mater*, with a little of the spinal cord or brain of a rabid dog, takes rabies with absolute certainty, and within a period seldom exceeding eighteen days. Thus we are saved the uncertainty belonging to subcutaneous inoculation, and from the weariness of the long incubation period. After this experiment the study of the disease went rapidly forward: it was proved that the virus existed in the nerves, and that by that route it travels from the original wound to the brain and spinal cord, and also that in some cases it can be conveyed by the circulatory system. It will be seen, therefore, that the manifestations of rabies may be varied, as the symptoms of the disease in the beginning will depend upon the particular region first encountered by the virus, and finally why it is that there are forms of the disease which until now were unrecognised, and which differ from the classic type. The operation of trephining is in itself harmless when performed with proper antiseptic precautions. If inoculation by trephining is performed on a series of rabbits from

I. Roux, Croonian Lecture, 1889.

the spinal cord or medulla oblongata of an animal which has just died, and this process be continued with successive animals, we find that the duration of the incubation period, which was at first from fourteen to eighteen days, gradually diminishes. It becomes shorter and shorter as the number of passages of the virus increases, until at the end of a hundred of the successive inoculations it has gone down first to seven days and finally to six. At that point it remains stationary, the rabic virus seeming to have attained its maximum virulence for that animal (the rabbit), and the virus is said to be "fixed."

In the year 1884, Pasteur had announced that the virus of rabies might be gradually attenuated by inoculation from the dog to the monkey, and then from monkey to monkey. The virus thus prepared no longer produces symptoms of rabies, but is able to protect against natural or artificial inoculation, where performed either hypodermically or submeningeally. However important such a discovery, it was not sufficient on which to found a practicable system of anti-rabic inoculation, and at the present time the protective material used by Pasteur is obtained from the "fixed" virus described above. The process for its preparation is similar in many respects to that already used for attenuating chicken cholera, rouget, and anthrax. A bottle, provided with an upper and a lower opening, is taken, the bottom covered with fragments of caustic potash, and the holes closed with plugs of cotton wool. A spinal cord is then placed in it, taken from a rabbit which has served in a passage series of inoculations. This cord, which contains a quantity of "fixed" virus, gradually dries, preserved from dust, and exposed to the contact of pure air, at a temperature of 25°C., care being taken to keep it at this point. If each day we take a small portion of this cord, and inoculate on the surface of the brain of a rabbit, we shall find that as the cord becomes dry in the sterilised warm air it loses its virulence. At the end of five days drying, it will only be capable of killing some few of the rabbits which receive it. At the end of fourteen days we find it absolutely innocuous, after having passed down the scale of gradually diminishing activity during the preceding days. Having now obtained our material containing the attenuated virus, if we each day inject subcutaneously into a dog a portion of the attenuated cord, crushed in water, taking care to begin the injection with the harmless fourteen days cord, to go on on the second day with the thirteen days cord, then the third day with the twelfth cord, and so on till we reach the cord of zero—or, in other words, spinal cord unattenuated, spinal cord which is deadly—this dog, thus attenuated, will not die, nay more, we may try him with the most active rabic virus inoculated into the brain, and he will remain perfectly well, though we know that otherwise intracranial inoculation produces rabies without fail. Here is, therefore, positive proof that the injections of the dried cord have produced exemption from the disease. The experiments may be repeated as often as one pleases, but the result remains the same. Dogs which have received subcutaneously the series of cords, commencing from the fourteenth day, cannot take disease either from the bites of mad dogs or in any other way. The protection has been obtained in a fortnight.

Rabies usually remains latent in a dog which has been bitten, for a period exceeding one month. M. Pasteur thought it might therefore be possible to profit by this long incubation period to give exemption from the malady before its manifestation. To elucidate this point, out of a number of dogs bitten by rabid dogs or inoculated under the skin with rabic virus, some were preserved for control experiments, while the others were subjected to the preventive inoculation of the dried cord in augmenting degrees of virulence. Of these latter not one took rabies, while of the former a great number died with the characteristic symptoms of the malady. It was proved possible to prevent hydrophobia after the bite.

In July, 1885, this system was applied to human beings, and up to 1893 14,430 persons had been treated at the Institut Pasteur at Paris alone. Of these 72 died, or one-half per cent. Since the treatment was commenced the mortality has steadily fallen, as is shown by the following table:—

Years.	Persons treated.	Deaths.	Mortality per cent.
1886	2,671	25	0·94
1887	1,770	14	0·79
1888	1,662	9	0·55
1889	1,830	7	0·38
1890	1,540	5	0·32
1891	1,559	4	0·25
1892	1,790	4	0·22
1893	1,648	4	0·24
Totals ..	14,430	72	0·50

Many attacks have been made on this method of antirabic inoculation, but into these I need not enter here They have in every case been proved to be based on misconception or without foundation, and if it has been shown that the treatment cannot cure the disease in persons who have recourse to it too late or who have been very severely bitten in the neighbourhood of the great nerve centres, the simple reply is that such claims have never been put forward for it by M. Pasteur. But that the system is of the very highest value, and that it does cure in a very large proportion indeed of cases, is shown by the above figures, and was freely acknowledged by the English Commission (I.) sent to enquire into it.

The most remarkable point, however, in the whole discovery of this preventive inoculation against rabies is that it has been carried out, the virus itself being still unknown. Not only do we not know how to cultivate it outside the body, but in allowing it to be really a microbe we can but do so by analogy, for as yet no one has been able to isolate it. Notwithstanding this, however, it is daily being attenuated and made to pass through the various stages of virulence. Unable to cultivate the organism artificially in flasks and tubes, M. Pasteur has been obliged to do so in the rabbit, and so easily and with such perfect regularity are these cultivations in the living animal performed that they are ready each day for use in the inoculations at a specified time and in the condition of genuinely pure cultivations. There is no stronger example of the power of the experimental method applied to medical matters than this one of the prevention of a malady, the absolute virus of which is still obscure. (II.)

Thus far, I have considered the work of Pasteur alone, but others have engaged in the same search with him, and though their experiments have not excited so much public interest as his, yet some present elements of the highest interest. In 1889, M. E. Högyes (III.) stated that dogs inoculated with a virus diluted to a certain degree with ·1 per cent. salt solution were protected against the disease. He used the fixed virus obtained by numerous transmissions through rabbits, and commencing with a very weak solution gradually progressed to the strongest, the pure cord. Dogs so treated are proof against inoculation with rabid material of every description, whatever the method of administration; that is to say, they are not only refractory to bites, but also to subcutaneous or even submeningeal inoculation, whether with ordinary virus from a dog dead of rabies or with the most powerful, *i.e.*, "fixed," virus. In this new method there is no more necessity for attenuated virus; the vaccin of M. Högyes is the "fixed" virus; no more inoculation with graduated viruses—the same virus serves for all, being simply diluted in more or less water. Attractive as this method appears on paper, it requires a very large number of experiments and its successful use by other workers before it can be accepted as established.

I. The Commission consisted of Sir Jas. Paget, Sir Jos. Lister, Sir Henry Roscoe, Drs. Brunton, Quain, Burdon-Sanderson, and Fleming, and Mr. Victor Horsley (Secretary).

II. Louis Pasteur: His Life and Work.

III. Nouvelle méthode pour prévenir la rage avant l'infection. Académie des Sciences de Buda-Pesth, 17th October, 1887.

In 1888, M V. Galtier (I.) communicated to the Académie des Sciences his discovery of the fact that intravenous injection of rabid material conferred immunity against the disease on herbivora, and that protection could be afforded even twenty-four hours after the subcutaneous injection of virulent medulla. It is not possible to go in detail into his experiments, but I have selected the following from amongst them :—

The experiment took place on the 13th December, 1887. A small piece of medulla was taken from a rabid dog, which had died three hours before, and rubbed down with 100° c.c. of sterilised water. A sheep was injected submeningeally with half a c.c. and died nine days later—rabid. Two other sheep received 2 c.c. subcutaneously, and shortly afterwards another 2 c.c., but in the jugular vein. Both were well on February 13th, 1888, two months later.

Two other sheep received 2 c.c. subcutaneously. Twenty-four hours later each received 2 c.c. in the right jugular, and five and a half hours after this 2 c.c. more in the left jugular. A dog inoculated on the brain on the 15th December, with the same virus, died rabid twelve days later. The sheep did not die, but on the contrary resisted hypodermic inoculation with very virulent virus, and continued healthy until the 12th April, when they were killed. Drs. Hellmann and Ferran, of Barcelona (II.) have somewhat modified Pasteur's method. They remove the brain of a rabbit dead of rabies on the eighth day, cut it in fragments, and dry it for 24 hours at 37°C. over caustic potash. One-third of this brain is crushed with the addition of some sand and 24 c.c of sterilised water, and after decantation 2 c.c. are injected into each person under treatment, night and morning, for a period of ten days. For the second vaccine the fresh brain of an eight days rabbit is similarly crushed with water, ten grammes of brain giving 30 c.c. of fluid. Each individual receives in all 40 c.c. of this fluid during the second period of treatment. Of 85 persons treated in this way no one has died.

The novelty of the procedure lies principally in the large quantities of brain emulsion injected, and it seems that the greater the quantity the more rapidly is immunity secured. In making these injections it is of first importance to introduce the fluid just under the skin and to avoid wounding muscles and nerves, as in such case instead of conferring immunity an acute attack of rabies may be determined. (III.)

Babes thinks that though Pasteur's method cures rabies from dog bites, it is not efficient in the treatment of wolf bites. Babes employs for this purpose injections of blood serum taken from dogs already rendered immune. He injects 20 grammes in the abdominal region on each occasion, and states that his method was successful in 24 cases.

Protopopoff kept virulent medulla in glycerine bouillon for 30, 46, and 60 days, and found that it lost its virulence, but was capable of giving immunity and of curing cases already affected, if injected under the skin or into the veins. Nineteen dogs were protected in this way, and of these ten were tested with the most virulent virus, both injected under the skin and under the *dura mater* All remained healthy. Protopopoff succeeded in preventing the disease developing even after intracranial inoculation !

These experiments, if confirmed, seem to promise a revolution in the methods of Pasteur.

Tetanus, Lockjaw. *Fr.:* Tétanos. *Ger.*. Starrkrampf, Tetanus.

Certain microbes—those for example, of anthrax—grow with such tremendous rapidity in the bodies of animals, that at the time of their death their blood contains more parasitic elements than blood-corpuscles. The bacilli occasionally form capillary obstructions and so act mechanically ; but like all living cells they have their vital products, and with such an enormous number of them it is easy to imagine that these very largely modify the nature of their surroundings. The bacillus of anthrax, which is specially greedy of oxygen, draws it from the blood-

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I. V. Galtier (Comptes Rendus, 16th April, 1888).

II. Sur la vaccination antirabique de l'homme (Gaceta medica Catalana, Vol. XI., No. 4, 1888).

III. Annales de l'Institut Pasteur, 1888, pp. 93 and 94.

corpuscles and produces asphyxia of the tissues; but the greatest source of danger from microbes is to be found in the poisonous products which they manufacture. A striking proof of this fact is given us by the diphtheritic bacillus, which, notwithstanding that it grows, not in the interior of the tissues, but on the surface of the mucous membrane—outside as it might be called, of the body—yet causes death, sometimes with fearful rapidity. In this case there is no invasion of the body nor struggle between the cells, but simply poisoning by the products of a very active parasite growing on the border of the false membrane. It is difficult to find in the body of an animal which has just died of an infectious malady these poisonous products of which we have been speaking, as the complex surrounding of tissues is unsuitable for such researches, and the poisons only being present in very small quantities, the animal during life partly eliminated them from its system; it is therefore in the cultivations in flasks and tubes that we must endeavour to find these products of the pathogenic activity of the microbes.

The first experiments on this subject were originated by M. Pasteur. He filtered a culture of cholera microbes through porcelain, and injected some of the germ-free fluid into a fowl, which became very ill, but finally recovered. We thus see that the chemical products obtained during cultivation of the microbe are able of themselves to cause the symptoms of disease, and it is therefore very probable that they are really manufactured by the microbe in the body itself of a fowl attacked by cholera. It has since been shown that many of the pathogenic microbes manufacture these poisonous products; and the microbes of typhoid fever, Asiatic cholera, blue pus, acute experimental septicæmia, and of diphtheria all belong to this class. The cultivations of the bacillus of diphtheria in particular become, after the lapse of a certain time, so charged with the poisonous substance that an infinitesimally small dose of it causes death to the animals, with all the characteristic signs observable after inoculation with the microbe itself, no one sign being wanting to complete the resemblance, even down to the gradual encroachment of paralysis when the dose has not been sufficiently strong to ensure a speedy death.

Tetanus, the disease of which I have now to speak, is similarly the result of the absorption into the system of poisons generated by a micro organism localised at some point of the animal body. Tetanus is produced by a minute anaerobic bacillus, first described by Nickolaier in 1884 as occurring in garden mould. It readily forms spores, and then appears, as a small rod six to eight micromillimetres in length, with a considerable swelling at one end, so that a sketch of it looks exceedingly like a drumstick. Nickolaier found that small quantities of garden mould produced tetanus in mice when placed under the skin, but was unable to isolate and grow the bacillus outside the body. Kitasato succeeded in this by a very ingenious procedure. He discovered the three facts, that the bacillus would not grow in presence of oxygen, that it readily formed spores, and that these successfully resisted high temperatures. He therefore took a small piece of tissue from the suppurating wound of a person who had died of tetanus, placed it in nutritive gelatine, and kept the whole in an atmosphere of hydrogen. Under the circumstances only the tetanus bacillus and one or two other anaerobic organisms developed. This was the first step in isolation. But the tetanus bacilli formed spores long before the others; as soon as this occurred Kitasato raised the temperature to 80°C for six hours. All other organisms (non-sporulating) were killed off, the tetanus spores alone remained (second step), and when planted in new media, sheltered from oxygen, soon produced abundant growths of bacilli.

In 1891 MM. Vaillard and Vincent (I.) gave a considerable amount of new information regarding tetanus. They showed that pure cultivations of the bacilli of tetanus, when washed free of the toxine with which they are normally associated, do not produce the disease, and they can only do so when the vital energy of the parts is co-existently lowered by injecting lactic acid, etc., or bruising the tissues. But they not only proved this, but also the converse, viz., that the toxine without the bacilli is able to cause the disease even when injected into the system in exceedingly minute quantities.

I. Vaillard and Vincent, Annales de l'Institut Pasteur, Jan. 1891, p. 1.

About the same time Behring and Kitasato announced that they had succeeded in protecting rabbits against tetanus by injecting small quantities of tetanus cultures, which had been treated with terchloride of iodine, under the skin. The injections were repeated several times, and the rabbits could then be inoculated with virulent tetanus cultures without in any way suffering. This was sufficiently remarkable, but a more surprising discovery soon followed. It was found that the blood serum of rabbits treated in this way acted as an antidote to tetanus cultures if injected before or along with them. Thus, mice which received a small injection of protective serum could be inoculated with a dose of virus one hundred times as great as that generally fatal, and yet experience no bad results.

Early in 1891, Behring gave a further account of his work. He stated that there were several ways of rendering rabbits immune. One is to commence by injecting a very small quantity of the virulent tetanus culture (non-fatal dose), and gradually increase the dose until it rises to double that generally required to determine death. Or, one can use larger quantities of culture—diminishing their excessive virulence by the addition of terchloride of iodine.

In March, 1891, Tizzoni and Cattani (Italian Archives of Biology) confirmed the statements regarding the antitoxic value of serum from immunised animals, "but," they add, "both in rabbits and rats we find that even when the tetanic intoxication has been produced by very small quantities of poison, and the injection of serum is made before tetanic symptoms appear (four hours after injecting the poison for instance), it is impossible to check or to arrest the development of tetanus."

Behring next proceeded to try (in company with Kitasato and Professor Schütz) whether it would not be possible to confer immunity on horses and the larger domesticated animals. His method is as follows:—Taking successive quantities of virulent cultures he adds to them varying proportions of terchloride of iodine, and begins the injections with that one containing most terchloride. At intervals of eight days the injections are repeated, until the blood, when injected into mice, gives immunity to tetanus. The horse then receives gradually increasing quantities of pure virulent cultivations. After each of the latter injections the protective power of the serum rises, and this protective power seems capable of almost indefinite development. Sheep are more easy to treat, but their blood does not acquire such marked antitoxic value. Shortly afterwards Kitasato, Brieger, and Wassermann published confirmatory experiments, but recommended a new method of conferring immunity, viz., to inject gradually increasing doses of a mixture of one part of sporeless tetanic culture, and two parts of thymus extract.

This work of Behring and Kitasato was very shortly followed by the appearance of a paper by M. Vaillard (1.), in which he describes a new and surer way of conferring immunity on rabbits, and one involving a much smaller mortality. He filters tetanus cultivations through porcelain, heats them to varying temperatures, and injects them under the skin. The first dose consists of 10 c.c. of germ-free culture, heated to 60°C. for one hour. This is repeated in three days' time. Five days later he gives 10 c.c. of the same fluid, which has only been heated to 55°C.; and in another five days 10 c.c., prepared for one hour at 50°C. This treatment gives immunity, and renders the blood antitoxic. Then follows the process of augmenting this antitoxic value by injecting, at ten day intervals, increasing quantities of fully-virulent filtured cultures. On the first occasion 5c. c., then 10 c.c., and so on up to 30 c.c. or more. This confers lasting immunity, so that animals immunised in 1890 and 1891 were still resistant to tetanus in April, 1892. Vaillard thus negatives Kitasato's contention that immunity can only be given by formed and not by soluble vaccins. And he further points out that the fowl (naturally immune to tetanus) yields a serum which is only antitoxic when the bird has previously received a considerable injection of filtered culture.

This antitoxic property of the serum requires a certain time to develop. Thus, if five or six days after injecting 20 c.c. of filtured culture into the

1. Vaillard, Annales de l'Institut Pasteur, 1892, p. 224.

peritoneum of a fowl one withdraws some blood from a vein, and treats with this blood a small animal like a mouse, the latter dies of tetanus. But in eight to twelve days the serum no longer kills—on the contrary, it protects against tetanus, and in eighteen to twenty days this protective action has reached a maximum. After four to six months it declines, but can always be renewed by a fresh injection of filtered tetanus culture into the body of the fowl.

The same volume of the *Annales de l'Institut Pasteur* contains an account of two cases of tetanus in man treated by injections of antitoxic serum. Both patients died. Four other cases are, however, reported in which the persons recovered. Whilst at Alfort, in 1893, I saw two horses rendered immune against tetanus, and two against diphtheria, from which blood was from time to time withdrawn to furnish serum for the treatment of cases of tetanus and diphtheria. Such serum may now, I believe, be obtained by application to the Pasteur Institute.

The *Berliner Thierärztliche Wochenschrift* for the 5th January, 1893, contains some notes on Behring's methods, which have already been translated by the present writer (*Veterinary Record*, January 28th and February 25th, 1893). Amongst them is an account of an experiment on a sheep and on a horse, for the purpose of determining the curative value of serum from an animal rendered immune againstt tetanus The sheep was inoculated with virulent tetanus culture, and began to show symptoms on the fourth day, when 50 c.c. of protective serum were injected. The animal is stated to have shown marked improvement the same evening, and to have been completely cured on the tenth day. The horse was inoculated on the 16th September, 1892. On the 19th symptoms were developed. 400 c.c. of protective blood were then injected, serum not being available. Next day 300 c.c. more blood were administered, and on the 21st September 300 c.c. of serum. On this day the symptoms appeared stationary, and by the 27th the animal was again quite healthy.

The above notes are extracted from Behring's work (Das Tetanusheilserum; Leipzig, 1892), which was followed in February, 1893, by a paper from the pens of MM. Roux and Vaillard (I.), dealing very thoroughly with the discoveries in connection with treatment by antitoxic serum made up to that date. They question the conclusiveness of these experiments on the sheep and horse, pointing out that no control experiments were made, and that they themselves had often failed to give tetanus to sheep by the injection of 2 c.c. of a very virulent culture. The so-called cure may, therefore, have been nothing of the kind. In the case of the horse, they state that it had already undergone some protective treatment, and, as it often happens that whilst immunity is being conferred animals show slight and passing symptoms of the disease, it must by no means be deduced that in this particular case the appearances would not have disappeared spontaneously, even though the protective serum had not been injected. In preparing a material for protecting animals, Roux and Vaillard take a very virulent culture, which kills mice in doses of $\frac{1}{4000}$ of a c.c. This is filtered through porcelain, and the filtrate treated with iodine solution (Gram's). In immunising a rabbit, for example, one injects—

First day,	3 c.c.	toxine	and	1 c.c.	Gram's	solution.
Fifth	,,	5 c.c.	,,	,,	2 c.c.	,,	,,
Ninth	,,	12 c.c.	,,	,,	3 c.c.	,,	,,

Eight days later the animal's serum is antitoxic. It renders its own weight of toxine inactive. From this time onwards one injects increasing doses of toxine of full virulence every eighth day, thus—5 c.c., 10 c.c., 15 c.c., 20 c.c., 30 c.c., 40 c.c. With each injection the antitoxic value of the blood increases. But this requires time, and if a very large dose of toxine be injected it may completely neutralise all the antitoxine present, so that the blood becomes virulent and kills mice. In a few days, however, this is reversed, and the blood becomes strongly antitoxic. But in the meantime it will be seen that the animal's tissues have been saturated with toxine, and yet it has shown no signs of tetanus. We must therefore conclude that immunity depends not only on the presence of antitoxine in the

1. Roux and Vaillard (Annales de l'Institut Pasteur; February, 1893).

blood, but also, and perhaps in a much greater degree, on the tissues becoming accustomed to, or tolerant of, the presence of the tetanic poison. The method may be equally well applied either to guinea-pigs, horses, sheep, or oxen. Professor Nockard has protected a sheep and a cow by means of it. It is exceedingly interesting to note that Roux and Vaillard consider that the filtered cultures, which contain no bacilli whatever, are just as efficient in producing immunity, and in rendering the blood antitoxic, as is the culture plus the bacilli.

Immunity once conferred persists for long periods. Thus, rabbits wbicb were protected two years before by means of 50 c c. of iodised and 15 c.c. of pure toxine were still proof against doses of toxine which killed untreated rabbits. But, on the other hand, the antitoxic value of the serum (and, naturally, the resistance of the animal to disease) undergoes a gradual decrease as time passes, so that the serum of a rabbit, whose protective power was originally represented by 100,000, had in one month receded to 5,000, and a fortnight later again was only 2,000.

In addition to that in the serum, antitoxine is present in the serosity obtained by applying a temporary ligature to the base of the ear, to a slight extent in the aqueous humour, in the urine and saliva, and in large quantities in the milk, which Professor Ehrlich views as the active agent in determining hereditary immunity.

But this property of the fluids can seldom be successfully employed in treating tetanus once symptoms are developed. Roux and Vaillard showed that even when the blood of inoculated animals had been rendered strongly antitoxic, and even protective, by the action of protective serum, tbe disease could still continue. In one instance, blood withdrawn from a guinea-pig dead of tetanus was actually still protective for other guinea-pigs. Of seven cases in man, all treated with protective serum, every patient died, yet in each case the blood was strongly antitoxic right up to the time of death.

At first sight it might appear sufficient that an effective method of rendering animals immune had been discovered; but a moment's reflection will show that however great an achievement from the bacteriologist's point of view, this bas little practical value. Tetanus is not a common disease, and it can scarcely be recommended that means should be taken to vaccinate every animal against it, even though such a procedure were easy. The practitioner, therefore, naturally asks, What can be done for a case where symptoms of the disease already exist? The answer is at present scarcely satisfactory. Whilst an exceedingly small dose of protective serum, injected an hour before the virus, protects with certainty, that dose requires to be greatly augmented if the virus be injected simultaneously; and if the disease be already established it becomes necessary to use immense quantities of serum, even when the latter possesses the exceedingly high immunising power of 1 to 1,000,000, or even 1 to 10,000,000. After a certain time it becomes impossible to prevent a fatal issue.

Influenza. *Fr.*. Influenza, Maladie du Cheval, Epidemie rouge, Fievre Typhoide, Pleuropneumonie Contagieuse. *Ger.*: Brustseuche, Pleuropneumonia Contagiosa.

Amongst the first attempts to protect against this disease, which may be more particularly defined as the thoracic form of influenza, were those of Korpsrossarzt Hell. His experiments, which are by no means conclusive, are detailed in the *Zeitschrift für Veterinärkunde* for October, 1892. As this date shows, the subject is quite new, and up to the present comparatively little work has been done in connexion with it.

An outbreak of influenza occurred in the 15th Hussar Regiment, quartered at Wandsbeck, and it occurred to Hell, who was acquainted with Behring and Kitasato's work, that the injection of blood serum, taken from horses which had already suffered from the disease in 1888, 1890, and 1892, might protect other animals against attack. Blood was therefore withdrawn with the usual precautions into tall glass cylinders, which were surrounded by ice, the serum allowed to separate for 24 hours, and injected subcutaneously and intratracheally in quantities of 40 grammes into those animals wbich had not yet been attacked.

No reaction could be detected. The horses so treated remained in infected stables without in any case contracting the disease. In addition to the serum treatment, strict measures of disinfection were observed, and the stalls frequently watered with dilute solutions of lysol.

Kreisthierarzt Paul Toepper (I.) made a further series of experiments during October, November, and December, 1892.

He treated in all 60 horses, distributed in two lots. In each case the disease ceased to spread when the healthy horses had received injections of protective serum, although they were standing in the same stables with infected animals. He considers not only that the spread of disease may be checked by this means, but that already existing cases may be cured. My own opinion is that such statements should be received with the greatest reserve.

About the same time another article by Corpsrossärzte Pilz and Neuse (II.) appeared.

The horses of two batteries of artillery had become infected before Hell's procedure was adopted. The outbreak was then cut short, and no further cases occurred. In a third battery cases certainly appeared, but it is not clear whether they had not become infected before the treatment commenced. The disease broke out later in a cavalry regiment. In two squadrons in which the injections were practised, only one case of disease occurred, but this may have been due to most of the animals having passed through a previous attack. In a third squadron nine horses suffered even after inoculation. The experiment was made of inoculating the healthy horses of another squadron, and then placing two diseased animals amongst them. Twenty-three days later two inoculated horses became ill, nor were their symptoms in any way slighter than usual. Neuse's method consistcd in injecting 40 grammes of serum. Of the horses so treated, 31 per cent. became affected, whilst only 30 per cent. of untreated horses suffered.

In the April number of the same journal is a critique on Hell's method.

The writer considers that even though the blood of animals which have just recovered from influenza may contain certain protective principles, it is by no means proved that these exist in sufficient quantity to protect other animals when injected. This question can only be set at rest by repeatedly placing inoculated horses between diseased ones, and noting the results. It should also be noted that Hell's method can, at best, only give temporary protection.

In the May number Hell replies to his critics, and himself gives a series of results which seem to partially contradict his previous conclusions. He lays particular stress on the fact that protective serum should only be taken from those recovered animals which have recently been again exposed to the disease, because, as he points out, the antitoxin is only present in the blood in any considerable quantity when the specific organism has lately been received into the body of the refractory animal.

On the recommendation of Professor Siedamgrotsky, 15 horses were treated with serum, and were afterwards placed between affected animals. All escaped the disease.

Paul Toepper (III.) in July, 1893, gave a resumé of experiments on 90 horses. The animals were in three lots. In each case, as soon as the disease broke out. the sick horses were isolated, and the unaffected ones inoculated with 50 grammes of serum. In a few days the sick horses were again placed with the inoculated ones, but in no instance did the latter contract the disease. No disinfection was practised. Three of the sick horses which were treated with injections of 100 grammes of blood serum seemed to derive considerable benefit from the procedure. Eight foals were also inoculated, four with 100 grammes of serum, and four with 150. One of the former became affected with the disease, but the other seven resisted.

Lies and Bertram (loc. cit.) relate the results of 54 injections. In no instance did the inoculated animals suffer from influenza, though exposed to contagion.

I. Berliner Thierärztliche Wochenschrift, 12th January, 1893.

II. Zeitschrift für Veterinärkunde, vol. 5.

III. Berliner Thierärztliche, Wochenschrift, No. 29, 1893.

Further experience of this method is required before t can be unreservedly accepted as a means of prophylaxis. For my part, I cannot help thinking that until means are discovered of artificially producing the disease with certainty, protection by injection of blood serum cannot become of practical utility. The ability to inoculate the disease would probably enable us to produce blood serum of definite and constant protective value. At the present time it must always remain doubtful whether the serum used has any protective quality, and if it has, to what extent that quality is developed.

Actinomycosis.

Though the following notes do not strictly belong to the subject of Animal Vaccines, they are suggestive and perhaps admissible.

Billroth (I.) reported a case of diffuse infiltrated actinomycosis cured by 15 injections of tuberculin.

The next case (II.) will be found in a more extended form in the *Veterinary Record* of October 29th, 1892, for which it was translated by the present writer. The subject was a servant nineteen years of age, who was suffering from a swelling of the left side of the face, caused by the presence of actinomyces.

The treatment adopted was to inject small doses of an extract prepared from the staphylococcus pyogenes aureus. Each injection was followed by a slight local reaction. After 25 injections the fistulæ had healed, the swelling diminished, and the patient had lost the difficulty in opening the mouth from which he had previously suffered. Dr. Ziegler states that he has had similar results from the employment of tuberculin.

But since Nockard and Thomassen in 1885 recommended the use of iodide of potash in this disease, its value has been recognised on all sides, so much so that, for the present at least, no bacteriological product seems likely to displace it.

Contagious Pleuro-pneumonia of Cattle. *Fr.*: Peripneumonie Contagieuse. *Ger.*: Lungenseuche.

For many years past the practice has prevailed of inoculating oxen in the tail with the clear amber-coloured serous exudate from the lungs of an animal which has just been slaughtered for pleuro-pneumonia. In France there are four methods of inoculation: hypodermic injection, subcutaneous inoculation with a hollow needle, sub-epidermic inoculation with the lancet, and intravenous inoculation. The latter, however, is not without danger, and sometimes results in the animal's death.

Of the methods enumerated, the first is that generally adopted in this country. It is sometimes modified by introducing threads soaked in the lymph under the skin of the tail. In from seven to fourteen days after inoculation the tail becomes the seat of inflammatory changes, which in most cases persist for from four to nine days, and then gradually decline. When all goes well they have disappeared after a further period of from fourteen to twenty-one days. A gangrenous process sometimes results, which necessitates amputation of the tail.

The significance and value of the operation have of late years been subjects of the keenest controversy, and owing to the peculiar circumstances of the case it has been difficult to give direct proof either of the efficacy or inefficiency of the procedure. I therefore consider that I cannot do better than quote the opinions of the Departmental Committee appointed by the Lords of the Privy Conncil in 1888 to enquire into the subject:—

"(1) From the evidence laid before us, it appears that cattle have been known to contract the disease after apparently successful inoculation.

"(2) In the dairies of London and Edinburgh, where inoculation is largely practised, the life of the animal is in general so short that it affords us no reliable knowledge as to the immunity from disease conferred by inoculation, and in cases where the animal is released from confinement we have very vague information as to its subsequent career.

I. Billroth, Wiener medicinische Presse, 1891, No. 9, p. 350.

II. Münchener med. Wochenschrift 41, 1892.

"(3) But the point which above all others militates against our obtaining an accurate idea of the exact prophylactic value of inoculation is the fact that it is not generally practised in this country, except in cases where an outbreak of pleuro-pneumonia has already occurred. The mode of treatment adopted under such circumstances is as follows :—The animals which are diseased, or suspected of being diseased, are slaughtered, whilst the rest of the herd are subjected to inoculation. It is, therefore, impossible to determine what effect, if any, inoculation has in suppressing such an outbreak in this country, inasmuch as in a large majority of cases we cannot be certain that after the slaughter had been carried out any diseased animals were left on the premises which would be likely to continue the disease. It might, therefore, be open to assumption that, even if inoculation had not been practised, the spreading of the disease would have been equally checked by the slaughter of the diseased or suspected animals."

Pasteur has suggested another method of obtaining protective material, viz., to inoculate a calf in the loose cellular tissue behind the shoulder with virus taken from the lung. Considerable ædema results, and a large quantity of material is obtained which is not so likely to be contaminated with accidental organisms as that taken from the lung. This material was employed by MM. Germont and Loir in a large number of experiments in Queensland, and, it is said, with the most satisfactory results.

By making cultivations from the material obtained after scraping the cut surface of a pleuro-pneumonia lung, M. Arloing (1.), of Lyons, succeeded in isolating four different organisms, amongst them that one which he considers the *causa causans* of the disease, and which he terms pueumobacillus liquefaciens bovis.

It promptly liquefies gelatine, and can be cultivated with or without access of air. Injected into the lungs it produces villous false membranes on the visceral pleura and foci of chronic pneumonia, disseminated throughout both lungs. Intravenous injection produced death in ten hours, with marked congestion of both lungs; all the interlobular spaces were infiltrated with serosity, as in the recent lesions of pleuro-pneumonia.

In 1890 Professor Schütz, of Berlin, made some experiments in Magdeburg to determine the value of inoculation. The animals were treated in the usual way, and after a lapse of sixteen days were, together with four control animals, exposed to infection. The autopsies showed that the twelve inoculated oxen had remained healthy, whilst three of the four control animals took the disease.

After this another twelve oxen were inoculated in Löbnitz. One died during the experiment. The remaining animals were exposed to infection in varying forms for nearly nine months, and on post-mortem examination were in every case found free of pleuro-pneumonia.

In 1893 Arloing made two communications to the French Academy, in which he referred to his discovery of the bacillus liquefaciens bovis, and stated that he had succeeded in obtaining from bouillon cultures a fluid which he termed pneumobacillin. This material, when subcutaneously injected into animals suffering from chronic pleuro-pneumonia, produced a rise in temperature which is much more marked than that produced in healthy animals. He, therefore, hoped that it might prove valuable as a means of diagnosis.

Canine Distemper.

Although this disease has been known for a long period, I am not aware that the micro-organism which almost certainly causes it has yet been clearly recognised. Mr. Everett Millais has interested himself greatly in the matter, and has succeeded in isolating some six varieties of organism from the nasal discharge, one of which is a bacterium and the other five cocci. All grow freely on nutrient gelatine, and of the five cocci two liquefy the gelatine. Of these one resembles very closely the pneumo-coccus of Friedländer, and Mr. Millais is inclined to consider it as the true producer of the disease, especially as this particular germ has been found in the blood of every animal examined. He has, however, met with great difficulty in exactly describing it, as, after a certain

1. See Journal of Comparative Pathology and Therapeutics for translation, Sept., 1889, p. 305

time of growth on nutrient gelatine, it appears to undergo morphological changes. This experimenter has, however, discovered a very interesting fact, viz., that if a mixed growth be started from the nasal discharge and cultivated on gelatine for some weeks, transplanting to new soil six or seven times, the fluid culture so produced may be employed as a vaccine. It suffices to smear a small quantity of such a culture on the nose of a well-bred dog to bring about a mild and uncomplicated attack of distemper, after which the animal enjoys an immunity equal to that following the disease when contracted in the usual way. The vaccine does not "take" in mongrels, nor is *absolute* immunity conferred in any case, but only a similar degree to that produced by ordinary attacks; and Mr. Millais assures me that instances have occurred in his own experience where animals have naturally contracted the disease three times during a period of a few years. Recent cultures produce a virulent form of the disease, and this virulence may be augmented by a series of passages through the dog.

Glanders-farcy. *Fr.*: Morve. *Ger.*: Rotz.

The presence of bacteria in the pus and juices of animals suffering from glanders-farcy was first clearly recognised by Christot and Kiener (I.), who described a micrococcus and motile rods varying in length from two to ten micromillimetres; but the bacterial origin of the disease was only satisfactorily demonstrated by the work of Bouchard, Capitan, and Charrin (II.), who in 1881 obtained cultures (which were not pure, however) from the pus of an abscess in a glandered man, with which cultures they produced glanders in guinea-pigs and in an ass.

A little later Löffler and Schütz (III.) announced similar discoveries, and succeeded both in isolating the pathogenic bacteria and in making pure cultivations of them.

The organisms consist of little rods 2 to 5 micromillimetres long, and ·5 to 1·4 micromillimetres broad, *i.e.*, about the size of bacillus tuberculosis, but a little thicker, or, to use another illustration, from one-third to two-thirds as long as a red blood-corpuscle is wide. They are either straight or slightly curved, their ends are rounded, and they usually occur in pairs. They are distinctly motile.

Until recent years the belief that glanders-farcy was a comparatively curable disease was widely held, and in point of fact little doubt exists that a certain small percentage of cases would recover were it not that under existing conditions all affected animals are required to be reported and destroyed. Not only did this belief in the curability of glanders-farcy obtain wide credence, but Mayhew seems to have noted that animals which had once been the subjects of farcy and had surmounted the attack did in cases subsequently resist when exposed to fresh chances of infection. The correctness of this latter belief received somewhat striking corroboration during the course of some experiments made by Löffler and Schütz. Their object was to produce glanders by inoculation with pure cultures, and they found that in a certain old horse the disease remained perfectly localised, and the ulcers very early evinced a marked tendency to heal. The animal, however, was killed, and on making a post-mortem examination it was found that it had already, in all probability, been affected with glanders, and that this had been running its course for some considerable length of time, as not only were there old scars on the septum nasi but old caseous masses scattered through the lungs. We have here an exactly analogous condition to that recently observed by Koch in the case of guinea-pigs already affected with tuberculosis, on which he made the observations that led him to the discovery of his protecting fluid. (IV.)

I. Christot and Kiener, Comptes Rendus, 1846, Vol. LXVIII., p. 1054, and Receuil de médicine vétérinaire, 1868, p. 93.

II. Bouchard, Capitan, and Charrin, sur la culture du microbe de la morve, etc. (Bulletin de l'Académie de médicine, 27th Dec., 1882. See also Bonley's report, Ibid, 1883, p. 1239).

III. Löffler and Schütz, Ueber den Rotzpilz (Deutsche medicinische Wochenschrift, 1888, No. 52.)

IV. Sims-Woodhead, Bacteria and their products, p. 266.

The fact being once established that one attack of glanders-farcy protects from subsequent ones, attempts were soon made to produce a mild and benign form of the disease which would confer immunity from subsequent infection or inoculation, and though the possibility of doing so has not been finally proved, these experiments have resulted in the production of a very valuable diagnostic agent. In 1890 Sacharoff published the results of certain work he had done with this object. He showed that it was possible to prepare an attenuated virus by passing the disease from one cat to another in series. The virulence thus becomes greatly exalted for the cat, but diminished for horses; and a horse inoculated with a pure culture derived from the last cat merely suffers a local disturbance, after which it is proof against virulent cultures. (I.)

In 1891, Kalning, of Dorpat, and Hellmann prepared an extract of glanders bacilli in a manner analogous to that adopted by Koch in preparing tuberculin. The pure cultures were extracted with a mixture of glycerine and water, the extract sterilised by heat and filtered to get rid of the dead bacilli. This material when injected into horses was found to produce a marked febrile reaction and local tumefaction when the animal was suffering, or had suffered, from glanders. The reaction sets in in from four to twenty-four hours from the time of injection, though the usual period is about eight hours.

This discovery was followed by a large number of experiments to discover the most active and reliable preparation of mallein, and to test the value of the material as a diagnostic agent. Amongst others Preusse (II.), Gulzeit (III.), and Johne and Hueppe (IV.), have employed modifications of Kalning's method. Foth (V.) prepares a dry mallein by precipitating virulent bouillon cultures with alcohol and drying the deposit *in vacuo*. This preparation is now exclusively employed in the Austrian army on account of its constant composition and perfect keeping qualities. The material generally employed in France and in this country is Roux's. It is prepared as follows:—The bacillus is first rendered extremely virulent by passage through rabbits and then sown in Nockard's glycerine-peptone broth, in which it is cultivated at 35°C. for four weeks. It is then sterilised at 110° C., filtered through paper and concentrated *in vacuo* to one-tenth of its original bulk. This is "raw" mallein. For use, 1 c.c. is diluted with 9 c.c. of a ·5 per cent. carbolic solution, and of this mixture 2·5 c.c. are injected.

In May, 1892, Prof. Dieckerhoff and Dr. Lothes gave the results of a large number of experiments in the Berliner Thierärztliche Wochenschrift. They may be briefly summed up as follows:—Mallein produces fever in glandered horses. Broadly speaking, healthy horses show no fever, whilst those suffering from glanders may exhibit a rise of temperature varying from 2°C. upwards. This appears in from 8 to 9 hours after injection, reaches a maximum 4 to 6 hours later, and then steadily declines. If continued for more than 24 hours it becomes doubtful if the disease is glanders. It is very necessary to observe the animals' temperature for some days before inoculation so as to determine its medium, and not to inject if fever be present.

Inoculation produces the other symptoms of fever, viz., dullness, loss of appetite, rigors, stiffness in movement, etc.

In addition to the general symptoms, mallein also produces a local reaction. At the point of inoculation an œdematous painful swelling results, which may be as large as a dinner plate, but seldom larger. This may also occur in sound animals, but if it be pronounced it points strongly to the existence of glanders, even when the temperature only shows a moderate increase. A further form of local reaction is seen on post-mortem in the red inflamed surroundings of the glanderous pulmonary lesions.

At a meeting of the Société Centrale de Med. Vét. Sciences of the 10th and 24th November, 1892, Nockard gave the results of some 6,000 inoculations. Several

I. See Journal of Comp. Path. and Therapeutics, 1890, p. 185.
II. Berliner Thierärztliche Wochenschrift, 1891, No. 29.
III. Zeitschrift für Veterinärkunde, 1892, No. 4.
IV. Sächsige Veterinarbericht, 1891.
V. Berliner Thierärztliche Wochenschrift, 1892, No. 45.

hundred animals reacted and were killed. In almost every case the indications of mallein were confirmed on post-mortem examination. In summing up the action of mallein as a diagnostic agent, I cannot do better than quote the conclusions expressed by Prof. McFadyean in the Journal of Comparative Pathology and Therapeutics for Jan. 1893 (p. 56). "The very numerous observations which have now been published at home and abroad regarding the action of mallein, appear to warrant the following conclusions :—

"(1) If in any horse that presents symptoms exciting a suspicion of glanders or is known to have been exposed to the infection of glanders, an injection of mallein raises the temperature from about the normal (under 101°F.) to 104°, and produces a marked inflammatory reaction at the seat of injection, that horse may be pronounced 'glandered.'

"(2) If in such a horse an injection of mallein determines a marked local reaction and an elevation of temperature equal to 2°F., that horse may be pronounced 'probably glandered.'

"(3) In horses that have already a febrile temperature (102°F. or over) the temperature reaction is inconstant and unreliable as a guide to diagnosis."

In February, 1893, Prof. Pilarios, of Athens, made a communication to the French Academy of Medicine on the therapeutic effects of mallein. He found that horses in the earlier stages of glanders were cured by injections of mallein repeated three or four times at intervals of eight days, "but," he significantly adds "in generalised glanders mallein on the other hand hastens death," and states that in some cases animals died from peracute glanders three to four days after injection. In a later communication (1894) he states that he has been successful in delaying, but not in curing, cases of pronounced glanders by injecting blood serum from animals rendered immune to glanders, but in this respect normal ox blood serum seems equally efficient. In slight cases the repeated injection of mallein is sometimes followed by a condition of non-reaction, and the animals can be considered cured.

Though exceedingly attractive, the above experiments do not strictly come within the limit of my subject, and cannot, therefore, receive further attention here.

Tuberculosis. *Fr.*: Tuberculose. *Ger.*. Tuberkulose.

The discovery by Villemin (I.) of the inoculability of tuberculosis, rendered the presence in the virulent material of an infectious agent like a bacterium very probable. That theory, which was at first the object of much opposition, received most striking confirmation from Robert Koch's discovery (II.) of the bacillus tuberculosis. The difficulty of recognising and isolating these very small and quite transparent bodies, contained as they are in liquids or tissues of the same refrangibility as themselves, formed an almost insurmountable obstacle to the study of the disease—an obstacle which Koch has succeeded in removing by dint of scientific methods and sustained exertion.

The bacilli of tuberculosis consist of little rods from 1·5 to 3·5 micromillimetres long, *i.e.*, from a quarter to a half the diameter of a red blood-corpuscle. The average width is more uniform, usually ·3 micromillimetres. The bacilli are either straight or slightly curved. They often show slight constrictions, which give them the appearance of a chain of ovoid beads.

At the inauguration of the International Congress of Medicine at Berlin in August, 1890, Dr. Koch announced the discovery of a material prepared from cultivations of tubercle bacilli, which, injected in certain small doses beneath the skin, conferred immunity against inoculation with tubercle bacilli, and cured tuberculosis in guinea-pigs even when very advanced.

I. Villemin, Causes et nature de la tuberculose (Bulletin de l'Académie de médicine, 1836, XXXII., p. 152 and 897), and Etudes sur la tuberculose. Paris, 1868, J. B. Ballière.

II. Koch, die Aetiologie der Tuberculose (Mittheilungen aus dem Kaiserliche mGesundheitsamte, II., 1884, p, 1.)

It was soon found that although it cured guinea-pigs, and produced improvement in cases of human lupus and tubercular disease of the joints, and of free surfaces, yet the substance in question did not fulfil the sanguine expectations which had been formed of it in the treatment of human and bovine tuberculosis; and at the present time its chief value to us as veterinary surgeons seems confined to the indications it gives of the presence of obscure or latent tuberculosis.

Tuberculin is prepared by extracting pure cultures of tubercle bacilli with glycerine. Koch gives the method in the *Deutsche Medicinische Wochenschrift* for the 22nd October, 1891. The bacillus is grown on sterile veal broth, to which has been added 1 per cent. of peptone and 4 per cent. of glycerine (Nockard's glycerine-peptone broth). After six or eight weeks the culture is evaporated to one-tenth of its bulk over boiling water and filtered through porcelain. The filtrate is "raw" tuberculin.

It is used as follows:—One-third of a c.c. of the material is injected into the loose connective tissue of the dewlap or shoulder, with antiseptic precautions. A distinct tumour forms at the point of inoculation, which is hot and painful. Examination of the lymphatic glands in the neighbourhood of the inoculation spot, and of tuberculous growths in other parts of the body, discovers them to be swollen, hot, and painful. There is also some general disturbance. In from nine to twenty-four hours, but generally between the twelfth and fifteenth hour, the temperature has risen from 1°C. to 3° or even 4°C. above normal. It remains at this elevation for three or four hours, when it begins to subside, and in forty-eight hours has generally returned to normal. When extensive tuberculosis exists, and the animals are feverish, the reaction is often slight or non-existent. From the result of a large number of experiments, Professor McFadyean (1.) seems inclined to regard 103°F. as the "critical" temperature. Any bovine showing symptoms of tuberculosis, or which has been exposed to the contagion of tuberculosis, and which exhibits a temperature of 103°F. or over after inoculation, may fairly be pronounced tubercular. It is foreign to the title of this paper to further follow the subject of tuberculin; but it is interesting to note that experimenters still continue the search for a method of anti-tubercular vaccination. Thus MM. Héricourt and Richet injected into the peritoneum or veins of four dogs graduated doses of avian tubercular material. The injections were repeated thrice, and the animals then successfully resisted inoculation with human tuberculosis. Four control (unprotected) animals died at intervals of eighteen, twenty-one, thirty, and forty-five days respectively. Continuing the experiment, nine vaccinated and twenty-one non-vaccinated dogs were injected with human tuberculosis. All the vaccinated animals resisted, whilst all the non-vaccinated, without exception, died. (Centralblatt für Bacteriologie und Parasitenkunde); see also Journal of Comp. Path. and Therapeutics, 1893, p. 183.

Immunity.

We have now briefly considered the steps by which the practice of protective inoculation has attained its present development. We have seen how the principle—that one attack of a specific infectious disorder protects against a second—attained in the hands of Jenner an extension and significance which we are only now beginning to appreciate, and that in demonstrating that immunity against smallpox resulted not only after recovery from smallpox, but also after cowpox, Jenner, whether wittingly or not, certainly sowed the seed which was afterwards to yield such a glorious harvest under the fostering care of Pasteur. We then passed in review the successive steps in the work of the latter, and learned how, whilst searching for analogous products to cowpox vaccin, he discovered that microphytes were no exceptions to Darwin's great law of adaptation to environment: that, given suitable conditions, their characteristics could be changed; their virulence extinguished or increased at will; that such modified qualities could be transmitted unchanged through many generations; and, finally, that when these attenuated growths were injected into the animal body they produced a passing fever, and after resistance to the disease.

1. Journal of Comp. Path. and Therapeutics, p. 143, 1893.

Such were the practical results of Pasteur's investigations—investigations which, I venture to say, have resulted in a greater advance in the prophylaxis of disease than had the collective efforts of all previous students of medicine from the advent of the Christian era.

The results are unchallenged; it now only remains to study and explain the mechanism by which they are brought about.

In considering the problem of acquired immunity, we have to answer the questions:

(1) What is immunity?
(2) What is the material which confers it?
(3) What are the changes on which its production rests?

Immunity may be defined to mean the power which the animal organism possesses of so resenting the presence of a disease-producing microphyte as to protect itself against disease.

The first attempt to explain the artificial production of immunity was that of Pasteur. Watching his artificial cultures, Pasteur discovered that after a certain time the organisms ceased to grow; that whereas they had previously been distributed evenly throughout the fluid they now began to subside, and that presently they fell to the bottom of the fluid, forming a well-marked precipitate. If left sufficiently long it was found no results followed the transplantation of a little of this sediment into a fresh flask of bouillon, and Pasteur rightly concluded that the organism was dead. Why was this? The answer seems perfectly clear. Just as a plant ultimately exhansts the soil in which it grows, so these little microbes had exhausted the fluid where they had been planted, and had ultimately perished for want of nourishment. If the growth were transplanted to fresh bouillon before it had lain too long at the bottom of the flask, it was found to be just as capable of increase as ever. The parallel between the process in the flask and the process in the body seemed so obvious that in the then state of pathological knowledge Pasteur may well be forgiven for having used it to explain conferred immunity. His suggestion was that the growth of the organism in the animal body depends on the presence there of a certain peculiar pabulum, that this pabulum is consumed by the modified bacilli used as "vaccine," and hence, when virulent bacilli are afterwards injected, they are unable to multiply—in a word, they are "starved out."

But this theory also presupposed not only that the specific pabulum was exhausted, but that it was never renewed again, which is both contrary to all probability and to ascertained fact. Other experimenters soon pointed out that anthrax bacilli grew abundantly in bouillon made from the flesh of refractory animals and in their organs after death, whilst it has since been shown that tetanus bacilli can perfectly well exist in the bodies of animals which are absolutely proof to that disease, and that anthrax bacilli multiply freely in the anterior chamber of the eye of sheep which are fully protected against anthrax. This idea had therefore to be abandoned.

Very shortly afterwards Chauveau formulated his theory (since revived in a modified form), which was that immunity consists not in exhaustion of the soil, but in the addition of something to it. In a similar way to Pasteur, Chauveau had found that many micro-organisms form, during their existence, certain bodies belonging to the aromatic series of fatty acids and alcohols, and that these were inimical to the growth of the organism. One body so formed is phenol or carbolic acid. "These organisms," said Chauveau, "are killed, not by exhaustion of the soil, but by their own metabolic products, and perish, like the victims in the Black Hole of Calcutta did, from non-removal of the results of their vital activities."

It was soon proved that the bactericidal properties of these bodies is so feeble, and the quantity in which they are produced so small, that they could not have any appreciable action on growth in the animal body. But the carrying out of this idea, viz., that in the vegetation of the pathogenic organisms substances are produced which are inimical to their own development, eventually led, through a succession of steps in experimental research, to the better knowledge which we now possess. The first of these steps was, I think, the discovery by Messrs. Smith and Salmon that pigs can be protected against pig typhoid by inoculating them

with the liquid in which the germ of the disease has been cultivated. The same year a similar discovery was made by the late Dr. Wooldridge in reference to anthrax. This was a year later succeeded by the discovery of MM. Chamberland and Roux, that in guinea-pigs immunity from splenic fever was acquired by injection into the peritoneal cavity of anthrax cultures sterilised by heat. A more effective material is produced by filtration through porcelain.

But this vaccination does not depend on the vaccine directly killing the bacilli, but on its action on the animal tissues, for it has been found that the bacilli can still grow very well in the filtered fluid used as "vaccine." As this vaccine does not kill bacilli outside the body, its protective action must be due to its awakening an inhibitory or destructive power which was previously latent or feeble.

The main question becomes, then, What happens in the body when these germ-free fluids are injected? Though it had long been known that the blood possesses an extraordinary power of destroying septic organisms, Von Fodor was the first to draw marked attention to the fact by his paper on the power of the blood to destroy pathogenic bacteria. He showed that the bacillus anthracis when injected into the blood is destroyed with extraordinary rapidity, and Nuttall demonstrated that both defibrinated blood and serum possess a similar quality. The same authority found that if anthrax bacilli be sown in frog's lymph they quickly become granular, and then so pale that they cannot be made out without staining. Finally they are destroyed. This process can go on qnite independently of the action of leucocytes. Buchner afterwards extended these researches of his predecessors and corrected some mistakes. The experiments which I have here slightly indicated serve to show the possible existence of a chemical agency in the living organism—an agency apart from the cells—capable of destroying infective microphytes as rapidly and effectively as corrosive sublimate. Such an agent may protect either by destroying or inhibiting the development of the microphyte, or by antagonising its chemical action.

Emmerich found that by injecting small doses of virulent cultures of rouget into the veins of rabbits he could confer immunity to that disease. That is to say, such a condition of blood was produced that, whereas in unprotected animals the injection of a fatal dose was followed by death on the fifth day with a temperature of 100°, and the blood was found swarming with bacilli, in the vaccinated animal there was little or no disturbance, and in eight hours the bacilli had absolutely disappeared from the blood.

Then came the question, Was this destruction of the bacilli dependent on the manufacture of life products of bacilli, or of some antibiotic substance (alexin) in the blood? The answer was furnished by taking an immunised rabbit whose tissues contained no bacilli, though they possessed antibiotic properties. By subjecting the previously minced muscle substance of this rabbit to pressure, and filtering through porcelain, a material was obtained which, when injected into a susceptible rabbit, conferred on it an antibiotic state, so that if a sufficient quantity of this substance were injected immediately after a fatal dose of the virulent culture of rouget, the development of the disease was averted. A few years before this would have been attributed to the direct action of the microbic life products on the rouget bacilli, but this cannot be admitted when it is known that certain pathogenic microphytes continue to live in the animal body long after death, when the relative quantity of their life products present must be enormous. Consequently there is only one possible alternative, viz.. that though the bactericidal substance is a chemical one, it is not identical with or contained in the substance introduced, but is what is termed in physiology a metabolic product.

These experiments were supplemented by those of Vaillard, Roux, and others (see previous sections), with tetanus and anthrax bacillus-free fluids. Another interesting fact was brought to light by their experiments, viz.: that a certain interval is necessary before any protective action results from the injection of filtered culture fluids, and this tends strongly to support the above view, viz: that the material contained in sterilised or filtered cultures does not of itself exercise the protective action against subsequent inoculation, but that it rouses to action certain dormant functions of the organism, thus acting as a kind of ferment or enzyme. Such a conclusion has been enormously strengthened by the work of Behring and Kitasato, who have brought into practical application the use of the

blood serum containing this protective material (antitoxin or alexin) and have shown that by previously injecting small quantities of such serum, complete immunity can be given against tetanus and diphtheria.

I may perhaps be excused for digressing here for a few moments to refer to some interesting researches of Hankin on the intimate nature of this protective substance. He considers that the production of the protective substance is intimately connected with a febrile reaction. He has shown that if rabbits are inoculated with very virulent authrax, no febrile reaction is set up, and the animals die rapidly. Inoculation with less virulent material is followed by a rise in temperature, and the animal resists for a longer time. If, then, fever is a sign that alexin is being formed in the blood, it should be possible, by producing such a reaction, to protect from otherwise virulent inoculation. This has been done by Buchner, who finds that rabbits may be protected against anthrax by simultaneously injecting pneumonia cultures, which set up the necessary febrile reaction.

"But," it may be said, "perhaps the increased temperature is of itself sufficient to kill the bacilli, without requiring the intervention of any metabolic product like alexin." Such a suggestion is, however, negatived by the observation made by Buchner that virulent bacilli could still be found in the body of a rabbit whose inoculation had been followed by a rise in temperature to 44°C., which would have been amply sufficient to attenuate, or even kill, the same bacilli when growing in a test tube.

The following experiment of Hankin's throws further light on the subject, and indicates another way of arriving at an explanation of the significance of fever. A rabbit was inoculated with anthrax, and 24 hours afterwards was found to have a temperature of 40·4°C. It was then decapitated, and its blood allowed to flow into alcohol. After some days the precipitated proteids were filtered off, dried, and extracted with water. The extract was filtered, and found to have the power of killing anthrax bacilli. In several other experiments Hankin succeeded in extracting a bacteria-killing substance from febrile blood. From normal blood, on the contrary, he could not by this method prepare a solution having bactericidal properties, except in one or two cases, and then only to a very slight degree.

The substances involved in this bactericidal action were named by Hankin "protective proteids," and his next experiments had for their object the further identification of their chemical constitution

The experiments of Salmon, Roux, Chamberland, Chantemesse, Widal, Wooldridge, Charrin, Ruffer, and others, had proved that immunity against various diseases could be produced by injecting the soluble life-products of microbes. These were generally considered to be ptomaines or animal alkaloids, but many considerations negative this view. For in the first place, if acquired immunity is of this nature, we are dealing with an acquired tolerance of a poison, which tolerance is conferred by a single dose, or at most a very few doses, of this alkaloid. Now, though tolerance of alkaloid does occur, it is limited in degree, and only obtained after a long succession of doses. Secondly, since acquired tolerance of this hypothetical poison results in the microbe being no longer capable of living in the body, this theory implies that the poison in question is produced by the microbe in order to enable it to live there. In other words, that it is a poison capable of lowering the bacteria-killing power possessed by every living animal body. Of course, a ptomaine might do this, though such have not been described; but certain albumoses produce a tolerance resembling that of microbic poisons. The first is the ordinary hemialbumose of proteid digestion, of which one minute dose protects against another for twelve hours. The second is the albumose of snake poison. Sewall injected a few minute doses of it into pigeons, and found that they were still protected from lethal doses three months after. These and other facts strongly suggest that immunity against disease is immunity against an albumose.

Secondly, we find that all poisons which are supposed to suppress the bacteria-killing power of the body are albumoses, or at least inseparable from them. Thus the juice of the papain tree, when injected into the body, allows the bacteria normally present in the intestine to wander into the tissues and produce a kind of septicæmia. The same is true of jequirity. Lastly, non-lethal doses of snake poison sometimes produce the same effect. Thus we see that the only

poisons known to have the power of aiding the growth of bacteria in the living body are not alkaloids but proteids. This fact gives further probability to the idea that not ptomanies but poisonous proteids are the substances concerned in aiding the entry of disease producing bacteria into the body, and consequently, when tolerance of them is acquired, of giving immunity against disease. Hankin isolated such an albumose from anthrax cultures, and was able to confer immunity on rabbits by injecting them with minute quantities of it, though any increase over the normal dose was inevitably followed by the death of the animal. Finally, he concludes that resistance to disease-germs depends on the presence of certain globulins whose increased formation is excited by the reception of the above-mentioned albumoses. If the globulins can be produced more rapidly than the poisonous albumoses the latter are neutralised, and the bacilli producing them are soon destroyed; if, on the other hand, the albumoses preponderate, the animal succumbs to their poisonous effect. Hankin also obtained a globulin from lymph glands which killed anthrax bacilli.

To resume: a third hypothesis which sought probably to explain smallpox vaccination was supported by Buchner and Wolffberg, though it is at the present time almost given up. I may term it the local hypothesis. It suggests that in the disease produced by vaccination the entire skin undergoes a change. In the vesicles the weakest cell-elements die, whilst the more vigorous survive; a suggestion which is supported by microscopic examination, inasmuch as one finds in the vaccinal vesicles both cells which have undergone coagulation necrosis, and others which are increasing in size and multiplying. After recovery from the disease only the more vigorous cell-elements remain, from which the new epidermis is formed. As the properties of the vigorous cell are inherited by its successors, the skin at the point where the disease organisms have been most numerous, only contains elements of the same character as could not be destroyed by the variolous contagion. Immunity is therefore supposed to be produced by the destruction of the weaker cells and the inheritance of acquired peculiarities, thus constituting it a particular case of Darwin's principle.

Another explanation of immunity is given by Ribbert. He terms it the inclusion theory (Umhüllungstheorie). The white blood-corpuscles are supposed to surround the bacteria, and prevent their forming poisonous products by cutting off the supply of oxygen necessary for growth.

Lastly, we have to consider the theory of phagocytosis advanced and supported with such skill by Metschnikoff. The basis of this theory is most lucidly and completely set forth in M. Metschnikoff's work on Inflammation (1.). He found that any injury to the animal body was at once followed by the passage of leucocytes through the walls of the neighbouring vessels (diapedesis), and their advance from all sides towards the injured spot. Once arrived, the leucocytes were seen to approach foreign particles or bacteria, and to gradually engulf and digest them. When the number of bacteria was large, it was found that they might multiply more rapidly than the leucocytes could digest them, and thus might quickly advance into the surrounding tissues, despite the utmost efforts of the white blood-corpuscles; or it was found that certain bacteria possessed the power of paralysing the leucocytes, so that although these were present in considerable numbers they were no longer capable of enveloping the bacteria. Metschnikoff compares the scene to a veritable battlefield, in which the invaders are the microbes and the defenders the leucocytes. If the invaders triumph the diseased condition becomes general; if the defenders are victorious the bacteria are annihilated and recovery occurs. In the latter case the leucocytes which survive are evidently those best fitted to overcome the bacteria in this miniature warfare: they are better provided for the struggle for existence. This property, said Metschnikoff, is handed down to their descendants, hence the origin of acquired immunity. The opponents of this theory have, however, rather pertinently pointed out that leucocytes are not principally nor even largely descended from previous leucocytes, but are chiefly formed by certain special tissues, as the spleen, marrow of bone, etc.

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1. Leçon's sur la pathologie comparée de l'inflammation, Paris, 1892.

Metschnikoff explains the migration of leucocytes towards the scene of action, by suggesting that just as certain male plant-cells (oosperms) are attracted by the secretion by the female cells of acid solutions, so the blood-corpuscles are attracted to the inoculation spot by the peculiar chemical products of tbe microbe. In other words, the white blood-corpuscles are drawn towards the microbes by a "chemical sensibility" which Metschnikoff calls positive chemotaxis.

But (to revert to our illustration) if the degree of concentration of the acid be too great, the oosperms are repulsed. Just in the same way if the bacteria products are present in excess the leucocytes are no longer attracted towards the bacteria, but are driven off or at least paralysed in so far as their destructive powers are concerned. This Metschnikoff terms negative chemotaxis.

Inflammation, then, can no longer be viewed as a degenerative process, but as the means by which tbe animal body is guarded against the entrance of infection. And Metschnikoff attributes to the white blood-corpuscles and certain similar cells the exclusive rôle in ensuring this protection.

Applying this theory to the explanation of disease, we have seen the possible effects of a local infection. But this is the least difficulty. The great crux for all such hypotheses lies in the explanation of conferred immunity. Metschnikoff's suggestion is that after the repeated injection of small quantities of the specific poison, or "vaccine," the tissues, and especially the leucocytes, become "accustomed" to its presence, and are no longer incommoded when the bacteria are introduced in large quantities, so that they are able to approach and destroy the latter without difficulty.

But if this idea be carefully considered, it is seen to mean little or notbing, and is, in fact, only another way of stating an unexplained fact; though it certainly has one advantange—that, namely, of enabling us to explain by analogy the general cbaracter and duration of immunity. Viewed in this light, it appears to receive some support from the work of Ehrlich in connection with ricin and abrin, in which it was shown that a very great similarity exists between the process of immunising against bacteria and against the above-mentioned substances. Solutions of ricin produce a similar effect to small doses of bacterial toxins, *i.e.*, immunity against the injection of solutions of ricin may be produced by the previous injection of successive small doses. Ehrlich discovered an antitoxic body, which he termed "antiricin," in the blood of animals rendered immune to ricin.

This material continues to exist for comparatively long periods in the blood, and it was found tbat animals could be rendered immune by the injection of blood of other animals already immune to ricin, just in the same way as immunity against diphtheria can be given by injecting blood of animals protected against that disease.

But in spite of these analogies, immunity to bacterial products is quite different. If an animal is immunised against anthrax, or a man against smallpox, the next invasion by virulent microphytes is not accompanied by the reception of any poison; but the animal tissues check the multiplication of bacteria, and thus the production of the poison. An animal which has become "accustomed" to a particular kind of bacterial toxin is by no means necessarily in a position to prevent the growth of the bacteria which produce that toxin. This has been shown by Kitasato's experiments with tetanus. He treated mice and rabbits with increasing doses of filtered cultures, and was thus able to produce a certain habituation to the poison, but never immunity. The tetanus bacilli could always grow in the hodies of such animals. This, of course, disposes of the theory that acquired immunity solely depends on habituation to the poison.

Other objections are:—

That the rapidity and completeness of phagocytosis does not always correspond with the resistance of the animal to disease; and

That it is not clear whether the living virulent bacteria are taken up by the phagocytes, or only those which have undergone degenerative changes.

In a paper of this kind it is manifestly impossible to deal with all the objections which have been brought against the "cellular" theory. The latter certainly seems to explain a vast number of facts which are otherwise inexplicable. At the

present time the liveliest controversy wages between Metschnikoff and his school, who support the theory of phagocytosis, and Buchner and his disciples, who believe that protection is due to the qualities of the serum (humoral theory). Neither theory explains all the observed facts, and perhaps, as in many such cases, the truth will finally be found to lie between them. The blood-plasma and blood-corpuscles are so intimately connected that any effort to artificially isolate them necessarily introduces elements which completely vitiate the result. But it certainly seems that if the phagocytes do destroy the bacteria they must effect their purpose by some chemical substance which they secrete, and which is more or less soluble in the serum. If this be so, it might explain at once the contention of Metschnikoff that the formed elements of the blood destroy the bacteria, and that of the "humoralists" that the antitoxic substance is found in the blood serum. For as the white corpuscles in contributing to the formation of fibrin undergo a process of degeneration, it seems very possible that the peculiar antitoxic substance might be set free, and, dissolved in the serum, furnish that product on whose presence as a soluble chemical substance the humoralists chiefly rely to substantiate their views.

The entire subject is, however, receiving the most earnest attention of a large number of workers. Its successful solution may with confidence be looked forward to, and bids fair to form the basis of as magnificent advances in the field of therapeutics as did the discovery of attenuated viruses in that of prophylaxis.